# AMMONIA EMISSION FROM EXCRETA OF GROWING-FINISHING PIGS AS AFFECTED BY DIETARY COMPOSITION

**Truong Thanh Canh** 



Promotoren:	<b>Dr. ir. M.W.A. Verstegen</b> Buitengewoon Hoogleraar op het Vakgebied van de Veevoeding, In het Bijzonder de Voeding van Eenmagigen
Co-promotor:	Dr. ir. A.J.A. Aarnink Instituut voor Milieu- en Agritechniek (IMAG-DLO) Afdeling Vechouderijtechniek
	Dr. ir. J. W. Schrama Landbouwuniversiteit Wageningen (LUW) Departement Dierwetenschappen, Leerstoelgroep Gezondheidsleer & Reproductie BIBLIOTHEEK LANDBOUWUNIVERSITEIT WAGENINGEN

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#### Proefschrift

ter verkrijging van de graad van doctor, op gezag van de rector magnificus van de Landbouwuniversiteit Wageningen, Dr. C. M. Karssen, in het openbaar te verdedigen op woensdag 24 juni 1998 des namiddags te vier uur in de aula

 $\tilde{X}_{i}(y,y) = \int_{-\infty}^{\infty} dy \, e^{-\frac{1}{2}y} \frac{\partial y}{\partial y} \frac{\partial y}$ 

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#### Abstract

Canh, T. T. Ammonia emission from excreta of growing-finishing pigs as affected by dietary composition. Ammonia, volatilised from pig slurry decreases manure's fertiliser value. Furthermore, the deposition of ammonia emitted into the atmosphere may cause undesirable changes in aquatic and terrestrial ecosystems. At present, there is increasing interest in nutritional means to reduce ammonia emission. In pigs, nitrogen excreted via faeces is predominantly incorporated in bacterial protein, which is less susceptible to rapid decomposition. Nitrogen excreted via urine is mainly in the form of urea, which is easily converted into ammonia and carbon dioxide by the enzyme urease present in facces. In different experiments the effect of dietary factors on nitrogen excretion of pigs and on pH as well as ammonia emission from slurry were investigated. Increasing the level of non-starch polysaccharides (NSP) in the diet shifted nitrogen excretion from urine to faeces and reduced slurry pH. The latter was caused by an increase of volatile fatty acid formation in faeces and slurry during storage. Lowering dietary electrolyte balance (dEB; Na + K - Cl) and adding acidifying Ca-salts: CaSO<sub>4</sub>, CaCl<sub>2</sub> or Ca-benzoate instead of CaCO<sub>3</sub>, a common added salt in commercial pig feed, reduced the pH of urine and slurry. Reducing dietary crude protein (CP) and supplementing essential amino acids decreased the total and urinary nitrogen excretion. These changes in dietary compositions, causing a lower urinary nitrogen excretion and pH of slurry, resulted in a strong reduction of ammonia emission from slurry. Changing dietary composition to reduce ammonia emission did not influence animal performance. It is concluded that manipulating the dietary factors such as NSP, dEB, Ca-salts, and CP, influences ammonia emission from slurry, while maintaining a normal pig performance. Such this approach might be an economic way to reduce the environmental impact of pig farming.

Doctoral thesis, Wageningen Institute of Animal Science, Wageningen Agricultural University, Department of Animal Nutrition. P. O. Box 338, 6700 AH, Wageningen. Institute of Agricultural and Environmental Engineering, Department of Livestock Engineering (IMAG-DLO). P. O. Box 43, 6700 AA Wageningen, The Netherlands.

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### Propositions Stellingen

- 1. Minimising the emission of gases considered to be environmental pollutants such as ammonia by dietary composition, is a logical starting point for sustainable animal production. Dietary manipulation can reduce ammonia emission from pig slurry remarkably whilst maintaining normal animal performance. *This thesis*
- 2. Slurry ammonia originates from dietary protein. However, a high reduction of ammonia emission can be achieved by manipulating both protein and non-protein fractions of the diet. *This thesis*
- 3. To achieve the aim of ammonia emission reduction according to legislation at low cost, different techniques and solutions should be incorporated in an integrated approach. This thesis
- 4. Although the problem definition is different with regard to ammonia emission from manure between Vietnam and the Netherlands, the final objective is the same for both countries. *This thesis*
- 5. Of the total nitrogen ingested, about 30% is retained in the pig and about 70% is excreted in urine and faeces. Nutritionists pay 70% attention to the first part and only 30% to the second part. This may not be fair.
- 6. There may be three answers for each scientific question: my answer, your answer and the right answer.
- 7. To be happy at one's scientific work, one must be fit for it, have a sense of success in it, and face doubts and uncertainty about it.
- 8. Manipulation of dietary fermentable carbohydrates to reduce ammonia emission from pig manure can be beneficial for animal health.
- 9. There is no conflict between the Old and the New The conflict is between the False and the True. HENRY VAN DYKE
- 10. Animal welfare is a human welfare affair.
- 11. It is nice to be important, but it is more important to be nice.

**Truong Thanh Canh** -Ammonia emission from pig excreta as affected by dietary composition. Wageningen, 24 June 1998

Kinh tang ba me. Tang em va cac con than yeu Dedicated to my parents, Huong, Nhim and Got

## **CONTENTS**

CHAPTER 1	General Introduction	1
CHAPTER 2	Influence of dietary factors on nitrogen partitioning and compositions of urine and faeces of fattening pigs	15
CHAPTER 3	Influence of dietary factors on the pH and ammonia emission of slurry from growing-finishing pigs	29
CHAPTER 4	Dietary carbohydrates alter faecal composition and pH and ammonia emission from slurry of growing pigs	45
CHAPTER 5	Effect of non-starch polysaccharide-rich by-product diets on nitrogen excretion and nitrogen losses from slurry of growing-finishing pigs	65
CHAPTER 6	Effect of dietary fermentable non-starch polysaccharides from pressed sugar beet pulp silage on ammonia emission from slurry of growing-finishing pigs	79
CHAPTER 7	Influence of electrolyte balance and acidifying Ca-salts in the diet of growing-finishing pigs on urinary pH, slurry pH and ammonia volatilisation	93
CHAPTER 8	Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs	113
CHAPTER 9	General Discussion	129
SUMMARY	In English In Dutch	147 151
PUBLICATION I	LIST	157
ACKNOWLEDG	EMENT	161
CURRICULUM V	/ITAE	163

Chapter 1

# **GENERAL INTRODUCTION**

### **GENERAL INTRODUCTION**

#### **Problem definition**

In recent decades, increasing emphasis has been placed upon developing sustainable agricultural production systems in which animals play an integral part. Pig production is constrained not only by technical factors (feed supply, animal health, management and genetic make-up) but also by environmental and socio-economic factors. In many developed countries, farmers are turning their farms into specialised production systems. The production level per animal and the production of animal product per ha of land as well as the farm size have been increased considerably. However, this high intensification has recently witnessed a rapidly growing public concern about environmental pollution (Tamminga, 1992; Van 't Klooster, 1996). Ammonia emission from surplus manure seems to create one of the most serious environmental problems (Aarnink, 1997).

In most parts of the world, especially in developing countries, farmers want to reduce ammonia emission for another reason to keep nitrogen in manure as a valuable nutrient source for crops. Competition in international market has also stimulated certain agricultural exporting countries, for instance in Southeast Asia, to establish biological clean cropping systems, in which organic fertilisers such as pig manure are motivated instead of chemical fertilisers. It has long been known that large losses of nitrogen occur while manure is being stored or applied to arable land (Freney *et al.*, 1983). Volatilisation of ammonia is one of the main causes of these losses. Ammonia that is lost from manure not only represents a decrease in fertiliser value, but is also a source of air pollution. Deposition of ammonia from the atmosphere may cause undesirable changes in aquatic and terrestrial ecosystems. It enhances the acidification and nitrogen enrichment of the soil, influencing nature ecosystems (Freney *et al.*, 1983; Roelofs and Houdijk, 1991; Fangmeier *et al.*, 1994).

Ammonia is a normal gas in the nature, being formed from biological degradation of protein in soil organic matter, plant residues and animal wastes (Freney *et al.*, 1983). It is an alkaline compound that is lighter than air and soluble in water. The maximum allowable concentration is 25 ppm (Gustafsson, 1997). Emission from livestock production is responsible for almost 50% of the total ammonia emission, in which pig production accounts for about 13% (Table 1). In some parts of the world with highly intensive pig production, as in the Netherlands, pig production is responsible for more than 35% of the total ammonia emission (Oudendag, 1993, Heij and Schneider, 1995). In Europe, emission density (mass per unit area) is 2-9 times higher than in the other continents (Dentener and Crutzen, 1994).

The negative effects of ammonia from animal production systems have already led to legislation in some countries, particularly those in the European Union, in order to reduce the emissions of ammonia to an acceptable level (Den Hartog, 1992; Van 't Klooster, 1996).

Sources	Emission (Tg <sup>a</sup> N/year)
Anthropogenic	
dairy cattle	5.5
beef cattle, buffaloes	8.7
pigs	2.8
horses/mules/asses	1.2
sheep/goats	2.5
poultry	1.3
fertiliser	6.4
biomass burning	2.0
subtotal	30.4
Natural	
wild animals	2.5
vegetation	5.1
ocean	7.0
subtotal	14.6
Total	45.0

#### Table 1. Yearly global ammonia emissions (Dentener and Crutzen, 1994)

The Dutch government, for example, has set the goal of achieving a reduction of 70% of ammonia emission by the year 2005, compared to the emission in 1980 (Van 't Klooster, 1996).

Various techniques have been developed to reduce ammonia volatilisation from slurry in pig houses and after application in the field. At present, there is also interest in nutritional means to reduce ammonia emission. Formerly, dietary adjustment to pig requirements was aimed at maximising production performance without special concern about its impact on environment. Recent environmental constraints have forced to assess nutrient feeding not only in terms of nutrients retained in the animals but also in terms of the non-utilised fraction of nutrients ingested. Nutrition can substantially contribute to the reduction of nitrogen excretion or to changing the characteristics of excreta. The aim of the project described in this thesis was to reduce ammonia volatilisation from pig slurry not by a single solution, but by a combination of different approaches in feeding. The main condition of the project was that these solutions should not have any adverse effects on animal health and welfare or on the production results, nor give rise to any other negative environmental effects.

#### **Environmental effects of ammonia**

The impact of ammonia on the environment can be divided into direct and indirect effects (Amann and Klaassen, 1995). There is a direct interaction of ammonia with atmosphere. Ammonia, which penetrates into the clouds, greatly affects the natural cloud chemistry. In particular, the oxidation processes of sulphur dioxide by ozone and hydrogen peroxide are depressed. Because under normal circumstances, sulphur dioxide is oxidised in cloud droplets, this process facilitates the removal of sulphur dioxide in precipitation. The oxidation of sulphur dioxide by hydrogen peroxide is strongly pH dependent and negatively influenced by ammonium concentration (Apsimon and Kruse-Plass, 1990). A high deposition of ammonium from the atmosphere can cause acid rain, which negatively affects the vegetation. However, the direct effects of ammonia on vegetation and the environment are of less importance than the indirect effects (Roelofs and Houdijk, 1991; Amann and Klaassen, 1995). Nitrogen enrichment of normally nutrient-poor soils may lead to the disappearance of nitrophobic species (Roelofs, 1986). Changes in soil microflora result in a decrease of soil fertility. A high deposition of ammonium also causes leaching of potassium, magnesium and calcium of the soil and reduces their availability for vegetation (Roelofs and Houdijk, 1991). Nitrogen oxides and ammonia may be converted into nitric acid and thereby contribute to acidification of soil and water. This acidification, in turn, leads to forest decline (Breemen *et al.*, 1982; Matzner, 1992) and pollution of ground and surface water (Soveri, 1992).

#### Ammonia emission and fertiliser

Nitrogen in animal manure has been and still is used to maintain and improve soil fertility to produce crop for human and animal consumption. If the applied manure is worked directly into the soil and if the supply of nitrogen to the soil is in close balance with the uptake in the crop, there would be hardly any nitrogen loss (Jongbloed and Lenis, 1993). There are differences between countries in the supply-need relationship. Countries with a dense of animal population produce larger surpluses of nitrogen. This excess is mainly the result of imports of concentrates for animal feed (De Boer et al., 1997). The overproduction of manure and the protein-rich feeding make this balance to become greater positive. This surplus supply of nitrogen is finally emitted into the environment and/or accumulated in the soil (De Boer et al., 1997) causing deterioration of soil biomass and fertility, and pollution of the air and ground water. In some agricultural countries where animal production is less intensive, such as Vietnam, the supply-need relationship is negative. The losses of nitrogen through emission and leaching after application of manure on the field exacerbate this. This negative relationship is normally balanced by supplying nitrogen from chemical fertilisers. However, the use of artificial fertilisers results in an increase of the production costs. Therefore, to achieve a sustainable agricultural production, it is necessary to assess the possibilities for reducing loss of nitrogen from manure through emission of ammonia. This can be done not only by improving slurry application techniques but also by modifying slurry characteristics in such a way that nitrogen is retained in slurry.

#### Effects of ammonia on indoor air quality

The increased practice of keeping livestock confined indoors has caused concern about the purity of the air within animal buildings. Bacterial decomposition of excreta collected and stored beneath

5

slatted floors in enclosed buildings produces a number of gases including ammonia, carbon dioxide, hydrogen sulphide, methane and sulphur dioxide. It is becoming increasingly evident that the environment within these buildings is an important factor affecting the productivity and health of the animals as well as the stockman. Verstegen et al. (1976) noted an effect of added ammonia in confined pig houses on energy metabolism and utilisation of pigs. Malayer et al. (1988) reported that odorous gases, such as ammonia, in the air of environmentally regulated buildings might diminish the stimulatory influence of boars on the onset of puberty in gilts. Drummond et al. (1980) showed that in filtered room air plus 50, 100 or 150 ppm of aerial ammonia, there was an average reduction in daily gain of 12, 30 and 29% of pigs, compared to a control treatment (0 ppm added ammonia). According to various reports, aerial ammonia depresses pig growth and damages the upper respiratory tract (Doig and Willoughby, 1971; Curtis et al., 1975; Donham, 1991; Morrison et al., 1993, Robertson, 1994). From these investigations it can be concluded that high concentrations of ammonia inside pig buildings are related to the prevalence of lung diseases among the pigs. This raised the question of whether lung disorders of pig farmers might be related to the same factors. Donham (1991) determined an association between the ammonia concentration above levels of 7 ppm and health problems of pig farmers. Gustafsson (1997) reported that high concentration of ammonia in pig houses (above 25 ppm) cause irritation to the respiratory organs and to the eyes, and aggravate the negative effects of dust on the health of pig farmers.

#### Factors determining ammonia volatilisation

The flow of nitrogen through the animal is an integral part of the global nitrogen cycle. In the pig, most of the body nitrogen originates from protein nitrogen present in the feed. The ingested nitrogen is either lost from the body directly or used to synthesise the body proteins (Moughan, 1993). The pigs need proteins in terms of amino acids rather than total supply of proteins. In the digestive tract of pigs, ingested proteins are firstly hydrolysed to amino acids by animal protease. Amino acids, which are not absorbed, are subjected to bacterial degradation or biomass formation in the large intestine, and finally excreted in faeces as bacterial proteins. The absorbed amino acids that are not used for body protein synthesis are deaminated to volatile fatty acids and ammonia. This ammonia is finally excreted in urine in the form of urea. Thus nitrogen is excreted by animals as apparently undigested nitrogen in faeces and as urea in urine. Nitrogen excreted via faeces is predominately incorporated into bacterial protein, which is less susceptible to rapid decomposition. In contrast, nitrogen excreted via urine is mainly in the form of urea, which is easily converted into ammonia and carbon dioxide by the bacterial urease:

$$CO(NH_2)_2 + 3H_2O \xrightarrow{\text{wrease}} 2NH^+_4 + 2OH^- + CO_2 \Leftrightarrow 2NH_3 + 2H_2O + CO_2 \tag{1}$$

Urease bacteria are present in faeces and on soil floors. The conversion of urea will start as soon as urine comes into contact with faeces. Since ammonia is a gas at normal atmospheric temperatures and pressures, it may be expected that any ammonia present in the slurry will be easily volatilised into the air (Freney *et al.*, 1983).

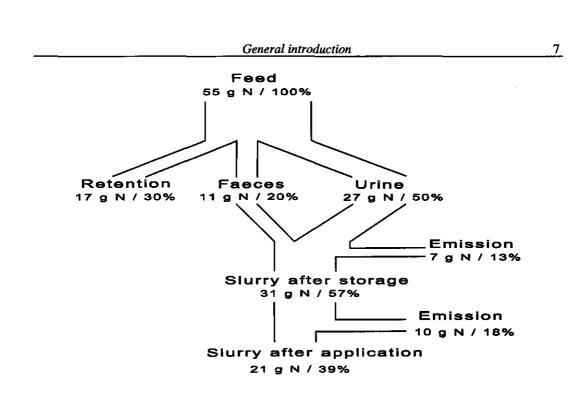


Figure 1. Nitrogen flow in growing-finishing pig production (Aarnink, 1997) The N intake is assumed to be 55g/ pig. day

Ammonia emissions from livestock systems occur at different stages in the production cycle. The nitrogen flow for growing-finishing pig production is visualised in Figure 1, as beginning with nitrogen intake by the pig and ending with the nitrogen uptake by the soil and losses by emission into the atmosphere. The highest ammonia emission occurs during application of slurry on the land (18% of N intake by the pigs). About 13% is lost during storage of slurry in the pig houses.

The high number and density of livestock population and their protein-rich feeding are the main causes of the high ammonia emission. The manner, in which slurry is collected, stored and distributed affects ammonia emission only as a consequence. Excessively protein-rich feed increases nitrogen excretion in urine, which apparently has a big influence on ammonia emission. However, there are large differences in urinary nitrogen excretion due to different factors such as animal, feed and environment. The most substantial excretion of nitrogen, about 60-70% of the total nitrogen excretion from pig farming originates from the growing-finishing pigs (Jongbloed and Lenis, 1993). Therefore, attempts to reduce ammonia emission should especially be focused on growing-finishing pigs.

Depending on different sources, there may be two types of factors influencing the rate of ammonia volatilisation. The first type, which is related to the liquid phase of production and availability of ammonia, are the slurry characteristics including the ammonium content and the pH of slurry. The second, which is related to the release of ammonia into the atmosphere, are the housing systems and indoor and outdoor climatic conditions.

#### Urinary urea and slurry ammonium

Most of nitrogen ingested by the pigs is excreted in urine (50%) and faeces (20%). The main origin of ammonia is urea in urine. There is a close relationship between total urinary urea nitrogen and total ammoniacal nitrogen in slurry. Aarnink (1996) found a linear proportional effect of the total ammonium nitrogen of the slurry on the ammonia emission. Smits *et al.* (1995) reported that ammonia emission in a dairy cow house reduced by 39% when the urea concentration decreased by 42%. In the slurry, ammonia (NH<sub>3</sub>) is in equilibrium with ammonium (NH<sub>4</sub><sup>+</sup>). The rate of ammonia volatilisation is determined by this equilibrium and by the rate of removal and dispersion of ammonia into the air.

$$NH_{3}(in the air)$$

$$\uparrow \qquad (2)$$

$$NH_{4}^{+}(in slurry) + OH^{-} \Leftrightarrow NH_{3}(in slurry) + H_{2}O$$

#### pH of slurry

The pH is one of the most important factors influencing the ammonia emission. Many workers have shown that ammonia volatilisation increases with increasing slurry pH (Stevens *et al.*, 1989; Hoeksma *et al.*, 1993, Sommer and Husted, 1995; Aarnink, 1997). The pH effect is to be expected from the equilibrium discussed in Equation 2. According to Srinath and Loehr (1974) the concentration of unionised ammonia (NH<sub>3</sub>) is a function of pH and the ionisation constants of aqueous ammonia,  $k_b$ , and water,  $k_w$ .

$$[NH_{3}] = [NH_{3} + NH_{4}^{*}] \times \frac{10^{pH}}{\frac{k_{b}}{k_{w}} + 10^{pH}}$$
(3)

or

$$\frac{[unionised \ ammonia]}{[total \ ammoniacal \ N]} = \frac{10^{pH}}{\frac{k_b}{k_w} + 10^{pH}} = F$$
(4)

Where F is the ratio between unionised ammonia and total ammoniacal nitrogen.

The amount of ammonia emitted is directly proportional to the concentration of the unionised ammonia. Higher pH values, therefore, favour the ammonia volatilisation. Aarnink (1996) reported a reduction of 10% of ammonia emission when the pH decreased by 0.1 unit. Many researchers have reduced the pH of slurry by acidification. Stevens *et al.* (1989) found that lowering the pH of slurry by 1 unit, from 7 to 6, by sulphuric acid reduced ammonia emission by about 82%. Although the effect of pH on ammonia emission is very strong, lowering the pH by acidification is not feasible because of the sophisticated techniques required and the high costs and, moreover, it may create other environmental problems.

#### Reduction of ammonia emission from pig husbandry

There are a number of ways to reduce ammonia emission from pig buildings. Covering slurry tanks was the first step in on-farm reduction of emission (De Bode, 1990; Williams and Nigro, 1997). To prevent ammonia volatilization from slurry under slatted floors inside the buildings, techniques have been developed for complete and frequent removal of slurry from pig houses (Hoeksma *et al.*, 1992). Air-cleaners, such as biofilters, are applied to some extent for cleaning the ventilation air from the buildings (Young *et al.*, 1997; Siemers and Weghe, 1997). The other techniques for reducing the release of ammonia from slurry are to use a liquid top layer (Derikx and Aarnink, 1993), urease inhibitor (Kemme *et al.*, 1993) and to treat slurry (Hoeksma *et al.*, 1993; Heber *et al.*, 1997). Recently, a new approach involving reducing the emitting surface area (slatted floor) of the slurry has been found to lower ammonia emission in pig houses to 50% at low costs (Aarnink, 1997; Verdoes, 1997). However, the reduction goal set for ammonia emission does not seem to be achievable by a single technique. There is a clear need for other approaches and additional measures in order to reach the goal laid down in legislation using economically feasible solutions. A combination of measures, therefore, will be needed.

#### Objective and outline of this thesis

The objective of the studies reported in this thesis was to investigate the effect of various dietary factors on nitrogen excretion pattern and slurry characteristics of growing-finishing pigs and their potential impact on ammonia emission. The ammonium content and pH of slurry seems to be the main slurry factors affecting the ammonia emission, which could be influenced by nutritional meas. The main condition in our investigations was that a normal performance of the pigs should be maintained while changing the diets for reducing ammonia emission. These solutions should not have any other negative effects on the environment. The two main feeding strategies used to obtain these reductions in this thesis are to reduce the urinary urea and the pH of slurry:

*Reduction of urinary urea*. Ammonia emission is positively related to the urinary urea or ammonium concentration of slurry (Freney *et al.*, 1983; Aarnink, 1997). There are two basic ways to reduce the urea or ammonium concentration of slurry:

- 1) Reducing the nitrogen content of the diet in combination with supplementing essential amino acids.
- Shifting nitrogen excretion from urine to faeces by increasing the amount of fibrous ingredients in the diet.

Reduction of slurry pH. The pH of slurry is a very important factor governing the ammonia emission (Stevens et al., 1989, Sommer and Husted, 1995). Reduction of urinary and (or) faecal pH will reduce the pH of slurry. Both approaches will be considered in our studies. Manipulating the dietary electrolyte balance and acidifying salts can influence the pH of urine. The pH of faeces can be influenced by manipulating dietary carbohydrates to increase the production of volatile fatty acids in the hind gut of pigs and in the slurry during storage.

#### Chapter 1

In Chapter 2 of this thesis, the effects of dietary factors on the partitioning of nitrogen excretion and slurry characteristics are qualified. Our hypothesis was that increasing the level of dietary non-starch polysaccharide (NSP) would increase faecal nitrogen excretion and decrease urinary excretion. Dietary NSP and electrolyte balance (dEB, Na + K – Cl) are also main factors influencing the pH of urine and faeces.

The ammonia emission from slurry of pigs fed the diets with different dEB and NSP levels is presented in Chapter 3. The effects of shifting nitrogen excretion from urine to faeces and lowering the pH of urine and faeces on the reduction of ammonia emission are shown.

In Chapters 4 to 6 the main ways of reducing ammonia emission from slurry by influencing the pH of faeces are presented. We hypothesised that fibrous feedstuffs in the diets of pigs would increase the volatile fatty acids (VFA) formation in the hind gut of pigs and in the slurry during storage resulting in a lower pH of faeces and slurry, and a lower ammonia emission. In Chapter 4, the effects of level and source of NSP on the pH and ammonia emission are presented. The effect of NSP-rich by-product diets on nitrogen excretion and nitrogen losses from slurry is described in Chapter 5. In this chapter, the feasibility of using the most common by-products in Vietnam instead of tapioca in pig feeding is discussed, in relation to the reduction of nitrogen losses from stored manure. In Chapter 6, the effect of a high fermentable source of NSP from sugar beet pulp silage on pH and ammonia emission is quantified.

The feasibility of reducing ammonia volatilisation by lowering the pH of urine is the subject of Chapter 7. The effects of reducing dEB and supplementing Ca in the form of acidifying Ca salts (CaSO<sub>4</sub>, CaCl<sub>2</sub> and Ca-benzoate) instead of alkaline CaCO<sub>3</sub> on the pH of urine and slurry and on the ammonia emission are quantified in this Chapter.

Lowering dietary protein can reduce the urinary urea excretion and the ammonia emission. In Chapter 8, the feasibility of reducing ammonia emission by optimising nitrogen utilisation in pigs by supplementing the diet with limiting amino acids and simultaneously decreasing protein content is presented.

In Chapter 9, the different approaches in feeding to reduce ammonia emission and their feasibility are discussed, not only in terms of nutrition but also in the light of sustainable agricultural production systems.

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### Chapter 2

# Influence of Dietary Factors on Nitrogen Partitioning and Compositions of Urine and Faeces of Fattening Pigs

T. T. Canh<sup>a, b, c, \*</sup>, M.W.A. Verstegen<sup>b</sup>, A.J.A. Aarnink<sup>a</sup> and J. W. Schrama<sup>b</sup>

<sup>a</sup>Department of Livestock Engineering, DLO Institute of Agricultural and Environmental Engineering (IMAG-DLO), Wageningen, The Netherlands.

<sup>b</sup>Wageningen Institute of Animal Science (WIAS), Departments of Animal Nutrition and Animal Husbandry, Wageningen Agricultural University, The Netherlands.

<sup>°</sup>Netherlands Foundation for the Advancement of Tropical Research.

<sup>\*</sup>Tel. (31) 317 476552; Fax: (31) 317 425670; E-mail: T.T.Canh@IMAG.DLO.NL

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# Influence of Dietary Factors on Nitrogen Partitioning and Compositions of Urine and Faeces of Fattening Pigs

T. T. Canh, M.W.A. Verstegen, A.J.A. Aarnink and J. W. Schrama

#### Abstract

An experiment was conducted to investigate the influence of dietary factors on the partitioning of nitrogen excretion and on the pH and composition of urine and faeces of fattening pigs. Sixteen male hybrid pigs of 80-90 kg BW were allotted to one of four diets: based on grains, by-products, grains plus tapioca or grains plus sugar beet pulp. Diets were formulated to have similar contents of NE and CP, and a similar lysine : NE ratio. Diets differed in non-starch polysaccharide content (NSP) and dietary electrolyte balance (dEB). During an 8-day period, urine and faeces were quantitatively collected daily in metabolism cages and mixed to a slurry. There was no effect of the diet on total nitrogen excretion (P > 0.05). However, the nitrogen excretion pattern differed between diets (P < 0.001). Pigs fed the by-product and the sugar beet pulp based diets excreted less nitrogen via urine and more nitrogen via faeces than pigs fed the grain and tapioca based diets. The type of diet significantly affected the pH of urine, faeces, and slurry. The pH of slurry from pigs fed the sugar beet pulp based dietary NSP content reduced the pH of faeces and slurry. A decreased dEB reduced the pH of urine and slurry. We conclude that dietary NSP influence the pH of urine, faeces, and slurry.

Key Words: Pigs, Diet, Nitrogen Balance, Excreta Composition, pH

#### Introduction

Ammonia emission from livestock farming should be reduced to prevent undesirable changes in aquatic and terrestrial ecosystems (Apsimon and Kruse-Plass, 1990). In pig production the main part of ammonia emission originates from urea in urine (Muck and Steenhuis, 1981). Urea is converted into ammonia and carbon dioxide by the enzyme urease present in faeces (Stevens *et al.*, 1989). The ammonia concentration and pH of the slurry are important factors influencing ammonia volatilisation (Freney *et al.*, 1983). Urea excretion can be reduced by lowering the dietary nitrogen intake (Gatel and Grosjean, 1992). There is some evidence that nitrogen excretion is shifted from urea in urine to bacterial protein in faeces by including fibrous feedstuffs in the diet (Kirchgessner *et al.*, 1993). Furthermore, non-starch polysaccharides (NSP) may influence the pH of faeces and slurry by VFA formation (Farnworth *et al.*, 1995). The pH of urine can be influenced by dietary electrolyte balance (**dEB**) (Patience *et al.*, 1987).

Chapter 2

Therefore, a study was conducted to investigate the effects of dietary NSP content and dEB level originating from different ingredient sources, on:

1) Partitioning of N excretion between urine and faeces;

2) pH and composition of urine, faeces and slurry;

3) Ammonia emission from the slurry.

This paper reports on the effect of these dietary factors on N partitioning and on the pH and composition of urine and faeces.

#### **Material and Methods**

#### Animals and Housing

Sixteen commercial crossbred barrows, with an average initial BW of 81.3 kg, were randomly allotted to one of four diets. The barrows were housed individually in an environmentally controlled room on metabolism cages, which allowed the separate collection of urine and faeces. Sizes of the metabolism cages were  $1.5 \times 0.55$  m (length × width) and were made of galvanised steel with wooden slats. The 18-day experimental period consisted of a 10-day adaptation period to allow the pigs to become accustomed to the cages and to the new diets, and of an 8-day period during which urine and faeces were collected. Room temperature was kept at 20°C and relative humidity at 55%.

#### Diets and Feeding

There are different principles of approaches to diets for fattening pigs: - the traditional grain based diets as used in several countries: barley and wheat may be the main energy sources (Diet 1); - the traditional Dutch diets in which many by-products are used as energy sources (Diet 2). Further, two more diets were made (Diets 3 and 4). These two had a piglet diet as a highly digestible basic part. To this basic part, grains and tapioca (Diet 3) or grains and sugar beet pulp (diet 4) were added. The ingredient sources in the different diets are given in Table 1. The diets mainly differed in non-starch polysaccharides (NSP) and dietary electrolyte balance (dEB). The calculated nutrient composition of the diets is given in. The sugar beet pulp based diet (13.80%) and the tapioca based diet (13.53%). Dietary electrolyte balance (calculated as meq Na + K - Cl [Patience *et al.*, 1987]) was different between diets. Dietary electrolyte balance and Ca level were highest in the by-product based diet and lowest in the sugar beet pulp based diet (Table 2).

The pigs were fed 2.5 times the energy required for maintenance, which was assumed to be 294 kJ NE per kilogram of metabolic body weight ( $BW^{0.75}$ ; ARC, 1981). The crude protein content was similar in the four diets (15.6 - 15.9%).

18

Ingredient			Diet	
(% As fed basis)	Grain	By-products	Tapioca	Sugar beet pulp
Barley 11.5% CP	62.62		25.00	25.00
Maize 9 % CP			14.70	14.80
Wheat 10 % CP	20.00		13.50	13.30
Pea		10.00		
Sugar beet pulp				30.00
Potato protein	8.00		5.00	6.10
Soya bean meal		15.05		
Soya bean flour			11.50	5.50
Sunflower meal		14.77		
Tapioca		50.00	25.00	
Soya bean oil	1.20	2.30	2.50	2.45
Cane molasses	4.83	5.00		
Chalk	1.65	1.15	0.50	0.50
CaHPO <sub>4</sub>	0.45	0.40	1.00	1.00
NaHCO <sub>3</sub>	0.15			
Salt	0.10	0.30	0.30	0.30
Premix <sup>1</sup>	1.00	1.00	1.00	1.00
L-Lysine-HCL				0.05
DL-Methionine		0.03		

Table 1. Ingredient composition	of the experimental diets
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<sup>1</sup> The vitamin and mineral premix supplied per 1 kg feed: 9000 IU vitamin A, 1800 IU vitamin D<sub>3</sub>, 40 mg vitamin E, 3 mg vitamin K, 5 mg riboflavin, 50 mg ascorbic acid, 12 mg d-pantothenic acid, 30 mg niacin amide, 350 mg choline-chloride, 40 mg vitamin  $B_{12}$ , 1 mg folic acid, 0.1 mg biotin, 0.5 mg Co; 0.06 mg Se, 0.4 mg J, 80 mg Fe, 25 mg Cu, 44 mg MnO<sub>2</sub>, 73 mg Zn, 20 mg Tylosin.

Synthetic lysine was added so that the lysine : NE ratio was similar in each diet. Amino acid data were based on Dutch feeding standards (CVB, 1994), which are similar to ARC (1981). The diets met all the nutrient needs of the pigs. Feed was mixed with water (2.5 L/kg of feed) and was provided in two equal meals per day. No additional water was given.

#### Measurements

To avoid disturbing the animals during the 8-day collection period, pigs were weighed 4 days before and 1 day after this period, before the morning feed. Urine and faeces of each pig were collected separately and weighed twice daily and stored at  $-20^{\circ}$ C. Urine was collected in a bucket via a funnel below the cage. Faeces were collected in a plastic bag ( $15 \times 30$  cm) using the velcro<sup>®</sup> support system (Van Kleef *et al.*, 1994). A piece of glasswool was placed in the funnel and a piece of fine-meshed gauze was placed over the urine bucket, to trap particulate. The urine buckets and faeces bags were changed twice a day. The urine funnels were changed every morning and the amount of urine left in the glasswool was determined by weighing the glasswool.

Composition		Ι	Viet	
(as fed basis)	Grains	By-products	Tapioca	Sugar beet pulp
ME (kcal/kg)	3077	3134	3300	3302
NE (kcal/kg)	2162	2176	2383	2239
CP (%)	15.64	15.61	15.90	15.78
Dig.CP (%)	13.01	12.88	13.21	12.45
Ileal dig. CP (%)	12.07	11.38	12.26	11.03
Ileal dig. Lysine (%)	0.64	0.64	0.70	0.67
Ileal dig. Methionine (%)	0.25	0.23	0.23	0.22
Crude fat (%)	2.90	3.25	4.13	4.09
NSP <sup>1</sup> (%)	13.80	18.17	13.53	31.18
Crude fibre (%)	3.88	6.89	3.51	7.29
NDF (%)	15.00	13.32	11.73	21.10
ADF (%)	4.86	7.85	4.94	10.14
Water (%)	15.29	12.49	12.52	12.09
Crude ash (%)	4.51	6.77	4.62	5.34
Starch (%)	44.17	40.09	47.99	30.56
Sugar <sup>2</sup> (%)	3.69	3.62	0.31	0.96
Phosphorus (%)	0.40	0.45	0.50	0.47
Dig. phosphorus (%)	0.18	0.18	0.26	0.25
Sodium (%)	0.13	0.14	0.14	0.15
Calcium (%)	0.79	0.75	0.47	0.57
Potassium (%)	0.65	1.17	0.67	0.49
Chloride (%)	0.30	0.37	0.24	0.25
Magnesium (%)	0.13	0.21	0.13	0.14
Copper (ppm)	8.10	11.10	7.30	5.80
Linoleic (%)	1.29	1.46	1.92	1.87
$dEB^3$ (meq/100g)	13.77	25.70	16.42	12.22

Table 2. Calculated nutrient composition of the experimental diets	Table 2. Calculated	nutrient com	position of th	e experimental diet	S
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Non-starch polisaccharides

<sup>2</sup>Amount of free mono-saccharides and di-saccharides

<sup>3</sup>Dietary electrolyte balance (dEB; calculated as meq Na + K - Cl)

To prevent nitrogen losses by evaporation of ammonia, the pH of the urine was kept below pH 2 by collecting the urine in 50 mL of 25% sulphuric acid.

The urine and faeces from each animal collected in the first 5 days of the collection period were sampled and stored at  $-20^{\circ}$ C until the nitrogen balance analyses were performed.

The pH of urine and faeces was measured twice a day. During the last 3 days, urine and faeces were collected without adding acid to the urine. Urine was stored at -20°C and faeces at 4°C. After the collection period, they were mixed according to the original excretion ratio. The pH of this slurry was measured directly after mixing and a sample was taken for chemical analysis. The different diets were sampled while they were being weighed out during the 8-day period. The daily samples were pooled and then subsamples were taken for nutrient component analyses.

#### **Chemical Analysis**

All samples were analysed in duplicate. Faeces and urine were analysed for DM, ash, N, and CF according to AOAC (1990) procedures.

Ammonium-N in faeces and urine was determined spectrophotometrically according to NEN 6472 (NEN, 1983). Volatile fatty acids in faeces and slurry were measured on a Packard 427 gas chromatograph, equipped with a flame ionisation detector (Derikx *et al.*, 1994). The method used for carbonates in faeces and urine was essentially the same as that described by Amundson *et al.* (1988) using ethane as an internal standard. Urinary urea was determined according to Moore and Kauffman (1970) procedures. The pH was measured at room temperature (about 20°C) with a Hanna instrument glass electrode (model HI 8417) directly submerged in the urine, in diluted faeces (mixed with distilled water in a ratio of 1:4), and in the slurry. Data on NSP, starch, sugar, and minerals are from CVB (1994) in which data on chemical composition digestibility and feeding value of feedstuffs have been presented along with the references for determination of chemical composition.

#### Statistical Analysis

The individual pig was the experimental unit. Effects of diet (grain based, by-product based, tapioca based and sugar beet pulp based) on average daily gain, N intake, N retention, N excretion, apparent faecal N digestibility, and urinary N : faecal N were analysed by one-way ANOVA using the GENSTAT statistical package (Genstat 5 Committee, 1993). When the *F*-test showed a significant effect of diet (P < 0.05), means were separated by using LSD with a confidence level of 0.05 (Genstat 5 Committee, 1993).

#### Results

No health problems occurred during the experimental period and no feed refusals were observed. Although the pigs fed the sugar beet pulp based diet had a higher rate of gain than the pigs fed the other diets, differences were not statistically significant (P > 0.05) (Table 3). Nitrogen intake slightly differed among diets (P < 0.05). Total nitrogen excretion was similar for all four diets. However, the nitrogen excretion pattern, which is indicated by the ratio of urinary nitrogen to faeces nitrogen, differed considerably between diets. Pigs fed the by-product and the sugar beet pulp based diets excreted less nitrogen via urine than pigs fed the grain and tapioca based diets, but excreted more nitrogen via faeces. As a result, apparent faecal nitrogen digestibility was higher in the grain (85.3%) and tapioca (84.8%) based diets than in the by-product (79.0%) and sugar beet pulp (74.7%) based diets. Nitrogen retention in pigs fed the sugar beet pulp based diet was greater than in pigs fed the grain, by-product and tapioca based diets. No significant difference in nitrogen retention was found between the other three diets.

		Diet				
Variable	Grains	By-products	Tapioca	Sugar beet pulp	P	SEM
Number of animals	4	4	4	4		
Initial BW (kg) <sup>1</sup>	83.5	83.1	84.3	86.3	NS	1.84
Final BW (kg) <sup>2</sup>	91.6	91.2	92.1	97.0	NS	2.12
Weight gain (g/day) <sup>3</sup>	621	625	602	823	NS	65.0
N intake (g/day)	54.3*	52.2 <sup>ac</sup>	50.9 <sup>bc</sup>	54.9°	*	0.85
Faecal N (g/day)	7.96ª	10.96 <sup>b</sup>	7.72 <sup>ª</sup>	13.87 <sup>c</sup>	***	0.59
Urinary N (g/day)	30.0 <sup>a</sup>	21.5 <sup>b</sup>	26.8°	16.8 <sup>c</sup>	***	1.48
Total N excretion (g/day)	37.9	32.5	34.5	30.6	NS	1.70
Urinary N:faecal N	3.83ª	1.97 <sup>b</sup>	3.55°	1.21	***	0.26
Apparent faecal N	85.3ª	79.0 <sup>⊾</sup>	84.8ª	74.7°	***	0.99
digestibility (%)						
N retention (% of intake)	30.1 <sup>a</sup>	37.8 <sup>b</sup>	32.2°	44.1 <sup>d</sup>	*	0.28
N retention (g/day)	16.3ª	19.7ª	16.4ª	24.2 <sup>b</sup>	**	1.41

# Table 3. Weight gains and nitrogen intake, retention, excretion, and apparent fecal nitrogen digestibility of pigs fed different diets

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; NS not significant

<sup>1</sup>The pigs were weighed 4 days before the collection period

<sup>2</sup>The pigs were weighed 1 days after the collection period

<sup>3</sup>Calculated for a period of 13 days between the initial BW and the final BW

<sup>a,b,c</sup>. Different letters in superscript indicate significant difference (P < 0.05)

The chemical composition of urine and faeces from pigs fed the different diets is shown in Table 4. Between diets, the amount of urine and faeces differed significantly. The pigs fed the sugar beet pulp based diet produced more faeces and less urine than pigs fed the other diets. The diet had a profound effect on urinary urea concentration. The pigs fed the sugar beet pulp based diet excreted 22 - 37% less urea in urine than pigs on the other three diets. Ash and carbonate contents of faeces and urine were lowest in the sugar beet pulp based diet, and so was the pH of faeces and urine. The pH of slurry, measured directly after mixing of urine and faeces, was also influenced by the diet. The pH of slurry from pigs fed the sugar beet pulp based diet was 0.44 to 1.13 units lower than the pH of slurry from pigs fed the grain, by-product and tapioca based diets.

The total amount of VFA present in the slurry was higher in the sugar beet pulp based diet (4.21 g/kg) compared with the grain (2.29 g/kg), by-product (2.41 g/kg) and tapicca (1.14 g/kg) based diets. However, this difference was not statistically significant (P > 0.05).

#### Discussion

The main objective of our research was to evaluate the feasibility of reducing the urinary concentration of urea and the pH of slurry by dietary composition.

		Diet						
Component	Source	Grains	By- products	Tapioca	Sugar beet pulp	— P	SEM	
		(4) <sup>1</sup>	(4)	(4)	(4)		1 <b>-</b>	
DM (g/kg)	Faeces	336.3ª	342.3ª	334.3ª	213.0 <sup>b</sup>	***	14.71	
	Urine	32.5°	35.9b <sup>b</sup>	30.9°	31.3ª	**	0.77	
NH4-N (g/kg)	Faeces	0.67	0.76	0.66	0.66	NS	0.06	
	Urine	0.22 <sup>a</sup>	0.13 <sup>b</sup>	0.31 <sup>°</sup>	0.40 <sup>d</sup>	***	0.03	
Tot.N (g/kg)	Faeces	7.99ª	9.32 <sup>b</sup>	9.18 <sup>6</sup>	8.59 <sup>a</sup>	*	0.47	
	Urine	6.61ª	5.30 <sup>b</sup>	6.63 <sup>a</sup>	4.90 <sup>b</sup>	**	0.30	
Ash (g/kg)	Faeces	59.9ª	93.3 <sup>b</sup>	78.3°	35.2 <sup>d</sup>	***	3.49	
	Urine	11.4 <sup>ª</sup>	16.3 <sup>b</sup>	9.3°	8.5°	***	0.42	
CF (g/kg)	Faeces	197.1 <sup>ª</sup>	260.5 <sup>b</sup>	185.6°	150.5 <sup>d</sup>	***	4.40	
Urea(mmol/l)	Urine	195.2ª	157.0 <sup>b</sup>	196.1 <sup>ª</sup>	122.9°	**	10.99	
Carbonate (l/kg)	Faeces	2.11 <sup>a</sup>	1.83°	1.09 <sup>b</sup>	0.23°	***	0.23	
	Urine	0.15ª	0.19 <sup>6</sup>	0.12 <sup>c</sup>	0.11°	***	0.01	
Tot.VFA (g/kg)	Faeces	4.23	3.84	3.83	4.47	NS	0.33	
	Slurry	2.29	2.41	1.14	4.21	NS	0.91	
pН	Faeces	6.84ª	6.85ª	6.95°	6.47 <sup>b</sup>	*	0.10	
-	Urine	7.48ª	8.19 <sup>6</sup>	7.03 <sup>ac</sup>	6.77 <sup>°</sup>	***	0.19	
	Slurry	7.64ª	7.80 <sup>ª</sup>	7.11 <sup>6</sup>	6.67°	***	0.13	
Urine (g/day)	-	4530°	4053 <sup>⊾</sup>	4039 <sup>b</sup>	3435°	**	155.5	
Faeces (g/day)		1126"	1181*	838 <sup>b</sup>	1650°	**	110.8	
Slurry (g/day)		5656*	5234 <sup>ac</sup>	4877 <sup>60</sup>	5085 <sup>bc</sup>	*	185.5	

Table 4. Amount and composition of faeces, urine and slurry from pigs fed different diets

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; NS not significant

<sup>1</sup>Number of observation in parentheses

<sup>a,b,c.</sup> Different letters in superscript indicate significant difference (P < 0.05)

Urinary urea concentration and the pH of slurry are the most important factors influencing the ammonia emission from pig slurry (Muck and Steenhuis, 1981, Aarnink *et al.*, 1993).

#### Partitioning of Excretory Nitrogen

In the present study the diets were similar in NE and CP contents but were formulated from different ingredient sources and differed mainly in NSP content and dEB. The total nitrogen excretion was not influenced by the diets. However, the by-product and sugar beet pulp based diets enhanced faecal nitrogen excretion and depressed urinary nitrogen excretion compared with the grain and tapioca based diets. The difference in nitrogen excretion pattern seems to be related to the amount of NSP in the diets. However, the crude fibre content seems to be more related to the excretion pattern than the NSP content. Therefore, cellulose seems to be the most important NSP component influencing the nitrogen excretion pattern. This is in agreement with findings by Mroz *et al.* (1993) who found the highest faecal nitrogen

excretion in pigs fed cellulose, and the lowest value was noted in pigs fed pectins, while feeding hemicellulose gave intermediate results. Fermentable carbohydrates serve as energy source for microflora in the large intestine of pigs, while urea, secreted from the blood into the large intestine, serves as nitrogen source. A high-energy supply of fermentable organic matter to the microbes of the large intestine induces a high secretion of urea from the blood (Low, 1985) and a high microbial growth. When urea is transferred to the lumen of the large intestine, it is broken down to ammonia by bacterial urease and then used for microbial protein synthesis. This microbial protein is finally excreted in the faeces. The amount of urea secreted from blood into the large intestine increases when dietary fibre increases (Low, 1985), resulting in a reduced urea and ammonia content in the portal plasma (Malmlöf, 1985).

The synthesis of microbial protein causes less ammonia to be reabsorbed from the colon. As a result, nitrogen excretion shifts from urine to faeces (Morgan and Whittemore, 1988; Mroz *et al.*, 1993; Schulze *et al.*, 1993).

#### The pH and Composition of Urine and Faeces

The present results demonstrated that the NSP content and dEB of the diet affected the pH of faeces, urine, and slurry. The pH of faeces was lower in pigs fed an NSP-rich diet than in pigs fed a diet with a low NSP content. According to Sommer and Husted (1995) VFA and carbonate contents are important factors influencing the pH of the slurry. Spoelstra (1979) reported that VFA are mainly produced from faeces by microbial degradation of dietary fibre and by deamination of amino acids. Only a non-significant amount originates from urine. In pigs, crude fibber is digested mainly in the hind gut by anaerobic microbial fermentation, and it is assumed to be one of the main substrates for VFA production (Imoto and Namioka, 1978). Our results suggest that the dominant process in the hind gut of pigs was degradation of dietary crude fibre to VFA. A high crude fibre content in the sugar beet pulp based diet enhanced VFA production. Although the concentrations of the VFA in the faeces did not differ so much between the diets, the total amount, calculated by multiplying the concentration with the amount of faeces produced clearly differed (Table 4). This resulted in a lower pH of the slurry and, to a lesser extent, of the faeces. The amount of VFA in faeces and slurry showed large variations, which could be attributed to the variation in microbial activity and in absorption from the large intestine.

The decomposition of fermentable carbohydrates is accompanied by the formation of  $CO_2$ . At the same time bacteria require  $CO_2$  for synthesising acetic acid and amino acids (Allison, 1969; Imoto and Namioca, 1978; Spoelstra, 1979). Thus, an increased microbial activity in the hind gut of pigs fed the sugar beet pulp based diet together with low  $CaCO_3$  content of this diet may explain the lower concentration of carbonate in faeces.

When comparing the two diets with high dietary crude fibre content, the pH measured in faeces of the sugar beet pulp based diet was lower than the pH measured in the faeces of the by-product based diet. This may be explained by the higher lignin content of the by-product based diet, causing less degradation of the fibre (Table 4). In addition to these findings, it suggests that the high content of dietary Ca increased faecal excretion of CaCO<sub>3</sub>, consequently increasing the pH of faeces.

24

The pH of the urine was strongly influenced by diet. The dietary electrolyte balance (dEB) is important in that respect. The dEB is an important factor affecting the acid-base status in animals. The maintenance of a constant blood pH is very critical for normal body function and, therefore, renal excretion of H<sup>+</sup> and other electrolytes will alter if the diet changes in this respect (Patience *et al.*, 1987; Tucker *et al.*, 1988; Haydon and West, 1990). In this experiment urinary pH was lowest in the sugar beet pulp based diet, which had the lower dEB level, and highest in the by-product based diet, which had the highest dEB level. According to Tucker *et al.* (1988) any alteration in the relative amount of Na, K, and Cl in the diet will affect urinary pH. Patience *et al.* (1987) reported that an increase in dietary Na and K resulted in an increase in the pH of urine. Rose (1989) showed that an excess of dietary K reduces the net acid excretion whereas a depletion of dietary K increases the net acid excretion. In the present study, dEB levels were different by adjustment of dietary Na, K and Cl levels. Morgin (1981) concluded that the acid-base status of an animal is influenced by a combined action of Na, K and Cl rather than by individual elements.

Table 4 shows that dEB is probably not the sole factor influencing urinary pH. The tapioca based diet had a higher dEB (Table 2), but a lower urinary pH than the grain based diet. This may be due to the confounding effect of dEB level with the dietary Ca level. Calcium levels were higher for the grain and by-product based diets than for the tapioca and sugar beet pulp based diets. In this study, Ca was added to the diet in the form of CaCO<sub>3</sub>. The effect of CaCO<sub>3</sub> was confirmed by Kienzle *et al.* (1991), who found an increase in urinary pH of cats when the diet was supplemented with CaCO<sub>3</sub>. Haydon and West (1990) reported that decreasing dEB by substitution of CaCO<sub>3</sub> for CaCl<sub>2</sub> decreased the pH of urine and slurry.

The pH of the slurry followed the same pattern as that observed in the urinary as well as faecal pH. A high content of dietary NSP in combination with a low dEB level seem to be the main factors causing the reduction of the pH of the slurry from pigs fed the sugar beet pulp based diet. An additional factor seems to be the CaCO<sub>3</sub> content of the diet.

#### Conclusion

The excretion of nitrogen shifts from the volatilizable form in the urine to the less accessible protein form in the faeces by including non-starch polysaccharides in the diet of pigs. Non-starch polysaccharides also lower the pH of faeces and slurry. A lower level of dietary electrolyte balance decreases the urinary pH resulting in a lower pH of slurry. Such an approach might be an economical way to reduce ammonia emission from pig farming. However, more knowledge is needed to quantify the effects of the different dietary factors that can reduce slurry pH, which is a major factor affecting the ammonia emission.

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### Chapter 3

# Influence of Dietary Factors on the pH and Ammonia Emission of Slurry from Growing-Finishing Pigs

T. T. Canh<sup>a, b, c, \*</sup>, A.J.A. Aarnink<sup>a</sup>, M.W.A. Verstegen<sup>b</sup> and J. W. Schrama<sup>b</sup>

<sup>a</sup>Department of Livestock Engineering, DLO Institute of Agricultural and Environmental Engineering (IMAG-DLO), Wageningen, The Netherlands.

<sup>b</sup>Wageningen Institute of Animal Science (WIAS), Departments of Animal Nutrition and Animal Husbandry, Wageningen Agricultural University, The Netherlands.

<sup>e</sup>Netherlands Foundation for the Advancement of Tropical Research.

\*Tel. (31) 317 476552; Fax: (31) 317 425670; E-mail: T.T.Canh@IMAG.DLO.NL

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## Influence of Dietary Factors on Nitrogen Partitioning and Compositions of Urine and Faeces of Fattening Pigs

T. T. Canh, A.J.A. Aarnink, M.W.A. Verstegen and J. W. Schrama

#### Abstract

We investigated the effects of dietary factors on the pH and the ammonia emission from slurry of growing-finishing pigs. Sixteen male hybrid pigs (80 to 90 kg BW) were allotted to one of four diets: based on grains, by-products, tapioca and sugar beet pulp. Diets were formulated to have similar NE and CP contents and a similar lysine : NE ratio. They differed in non-starch polysaccharide content (NSP) and dietary electrolyte balance (dEB). Urine and faeces were daily collected quantitatively in metabolism cages and mixed as a slurry at the end of the collection period. After mixing, the pH and the ammonia emission from the slurry were measured daily in a laboratory set up for 7 days at 20°C. The type of diet affected the pH of the slurry and the ammonia emission (P < 0.001). The pH of the slurry from pigs fed the sugar beet pulp-based diet was 0.8 unit lower and ammonia emission was 52 to 53% lower than of the other three diets. The low dEB and high NSP, sugar beet pulp-based diet increased the volatile fatty acid (VFA) concentration and reduced the pH and ammonia emission from the slurry. We conclude that dietary NSP and dEB influence the pH and ammonia emission from slurry of growing-finishing pigs.

#### Key Words: Pig, Non-starch Polysaccharides, Electrolyte Balance, Slurry, Ammonia

#### Introduction

Ammonia emission from pig husbandry contributes substantially to environmental pollution (Apsimon and Kruse-Plass, 1990) and therefore should be reduced. Ammonia is mainly formed by enzymatic conversion of the urea in urine (Muck and Steenhuis, 1981). The ammonia concentration and the pH of the slurry are important factors influencing the ammonia emission (Freney *et al.*, 1983). Urea excretion can be reduced by lowering dietary N intake (Gatel and Grosjean, 1992). There is increasing evidence that nitrogen excretion is shifted from urea in urine to bacterial protein in faces when fibrous feedstuffs are included in the diet (Kirchgessner *et al.*, 1993; Canh *et al.*, 1997). Researchers interested in incorporating non-starch polysaccharides (NSP) into pig diets have proposed possible benefits of the end products (VFA) of fibre fermentation. Furthermore, VFA may influence the pH of faces and slurry (Farnworth *et al.*, 1995). The pH of urine can be influenced by the dietary electrolyte balance (dEB) (Patience *et al.*, 1987). So far, however, the concept of manipulating diets with NSP and dEB to reduce the pH of and the ammonia emission from slurry has not been thoroughly explored.

Therefore, we investigated the effects of type of diets differing in NSP and dEB on the pH and ammonia emission from slurry of growing-finishing pigs.

#### **Material and Methods**

#### Animals and housing

We used 16 commercial crossbred barrows with an average initial BW of 81.3 kg in a completely randomised design to evaluate the effect of dietary non-starch polysaccharides (NSP) and dietary electrolyte balance (dEB) on the pH and the ammonia emission from slurry of growing-finishing pigs. The barrows were housed individually in an environmentally controlled room in metabolism cages that allowed the separate collection of urine and faeces. The sizes of the cages were 1.5 m  $\times$  0.55 m (length  $\times$  width), and were made of galvanized steel with wooden slats. The 18-day sampling period consisted of a 10-day adaptation period to allow the pigs to become accustomed to the cages and to their new diets, and of 8 days during which urine and faeces were collected. Room temperature was kept at 20°C and relative humidity at 55%.

#### Diet and feeding

There are different approaches to the way diets are formulated for growing-finishing pigs. Traditional grain based diets are used in several countries. Barley and wheat are often the main energy sources, and Diet 1 was similar to these diets. Diet 2 was formulated using by-product ingredients, including tapioca and cane molasses, and as such represented a typical commercial ration employed by the Dutch industry. Further, two more diets were formulated (Diets 3 and 4). These two had a highly digestible pig diet as a basic part. To this basic part, barley and tapioca (Diet 3) or barley and sugar beet pulp (Diet 4) were added. The ingredients of the different diets are given in Table 1. The diets mainly differed in NSP and dEB. The calculated nutrient composition of the diets is given in Table 2. The sugarbeet pulp based diet had the highest NSP content (31.18%), followed by the by-product based diet (18.17%), the grain based diet (13.80%), and the tapioca based diet (13.53%). Dietary electrolyte balances (calculated as mEq Na + K - Cl [Patience *et al.*, 1987]) differed between diets. Dietary electrolyte balance was highest in the by-product based diet and lowest in the sugar-beet pulp based diet (Table 2).

The diets met all the nutrient needs of the pigs. The pigs were fed 2.5 times the NE required for maintenance, which was assumed to be 294 kJ NE/kg of metabolic  $BW^{0.75}$  (ARC, 1981). The crude protein contents were similar in the four diets (15.6 to 15.9%). Synthetic lysine was added so that the lysine : NE ratio was similar in each diet. Amino acid data were based on Dutch feeding standards (CVB, 1994), which is similar to ARC (1981). Feed was mixed with water (2.5 L/kg of feed) and was provided in two equal meals per d. No additional water was given.

Ingredient		Di	et	
(% As fed basis)	Grain	By-products	Tapioca	Sugar beet pulp
Barley 11.5% CP	62.62		25.00	25.00
Maize 9 % CP			14.70	14.80
Wheat 10 % CP	20.00		13.50	13.30
Pea		10.00		
Sugar beet pulp				30.00
Potato protein	8.00		5.00	6.10
Soya bean meal		15.05		
Soya bean flour			11.50	5.50
Sunflower meal		14.77		
Tapioca		50.00	25.00	
Soya bean oil	1.20	2.30	2.50	2.45
Cane molasses	4.83	5.00		
Chalk	1.65	1.15	0.50	0.50
CaHPO₄	0.45	0.40	1.00	1.00
NaHCO <sub>3</sub>	0.15			
Salt	0.10	0.30	0.30	0.30
Premix <sup>1</sup>	1.00	1.00	1.00	1.00
L-Lysine-HCL				0.05
DL-Methionine		0.03		

Table 1. Ingredient composition of the experimental diets

<sup>1</sup> The vitamin and mineral premix supplied per 1 kg feed: 9000 IU vitamin A, 1800 IU vitamin D<sub>3</sub>, 40 mg vitamin E, 3 mg vitamin K, 5 mg riboflavin, 50 mg ascorbic acid, 12 mg d-pantothenic acid, 30 mg niacin amide, 350 mg choline-chloride, 40 mg vitamin  $B_{12}$ , 1 mg folic acid, 0.1 mg biotin, 0.5 mg Co; 0.06 mg Se, 0.4 mg J, 80 mg Fe, 25 mg Cu, 44 mg MnO<sub>2</sub>, 73 mg Zn, 20 mg Tylosin.

#### Measurements

To avoid disturbing the animals during the 8-day collection period, the pigs were weighed 4 days before and 1 day after this period, before the morning feeding. The urine and faeces of each pig were collected separately and weighed twice daily. The urine was collected in a bucket via a funnel below the cage. The faeces were collected in a plastic bag  $(15 \text{ cm} \times 30 \text{ cm})$  using the velcro<sup>®</sup> support system (Van Kleef *et al.*, 1994; Canh *et al.*, 1997). The urine and faeces collected from each animal during the first 5 days of the collection period were used for nitrogen balance analyses (Canh *et al.*, 1997). The urine and faeces at 4°C. After the collection period, urine and faeces were mixed according to the original excretion ratio. The pH of this slurry was measured directly after mixing, and a sample was taken for chemical analyses and in vitro measurement of the ammonia emission. The different diets were sampled while they were being weighed out during the 8-day period. The daily samples were pooled and then subsamples were taken for nutrient component analyses.

Composition			Diet	
(as fed basis)	Grains	By-products	Tapioca	Sugar beet pulp
ME (kcal/kg)	3077	3134	3300	3302
NE (kcal/kg)	2162	2176	2383	2239
CP (%)	15.64	15.61	15.90	15.78
Dig.CP (%)	13.01	12.88	13.21	12.45
Ileal dig. CP (%)	12.07	11.38	12.26	11.03
Ileal dig. Lysine (%)	0.64	0.64	0.70	0.67
Ileal dig. Methionine (%)	0.25	0.23	0.23	0.22
Crude fat (%)	2.90	3.25	4.13	4.09
NSP <sup>1</sup> (%)	13.80	18.17	13.53	31.18
Crude fibre (%)	3.88	6.89	3.51	7.29
NDF (%)	15.00	13.32	11.73	21.10
ADF (%)	4.86	7.85	4.94	10.14
Water (%)	15.29	12.49	12.52	12.09
Crude ash (%)	4.51	6.77	4.62	5.34
Starch (%)	44.17	40.09	47.99	30.56
Sugar <sup>2</sup> (%)	3.69	3.62	0.31	0.96
Phosphorus (%)	0.40	0.45	0.50	0.47
Dig. phosphorus (%)	0.18	0.18	0.26	0.25
Sodium (%)	0.13	0.14	0.14	0.15
Calcium (%)	0.79	0.75	0.47	0.57
Potassium (%)	0.65	1.17	0.67	0.49
Chloride (%)	0.30	0.37	0.24	0.25
Magnesium (%)	0.13	0.21	0.13	0.14
Copper (ppm)	8.10	11.10	7.30	5.80
Linoleic (%)	1.29	1.46	1.92	1.87
$dEB^3$ (meq/100g)	13.77	25.70	16.42	12.22

#### Table 2. Calculated nutrient composition of the experimental diets

<sup>1</sup>Non-starch polisaccharides

<sup>2</sup>Amount of free mono-saccharides and di-saccharides

<sup>3</sup>Dietary electrolyte balance (dEB; calculated as meq Na + K - Cl)

The ammonia emission was determined in vitro in a laboratory set up at 20°C for 7 days following the procedures described by Derikx and Aarnink (1993). Slurry (2 kg) was placed in a vessel with an inside area of 284 cm<sup>2</sup> covered by a lid. Air entered the vessel through small holes at the edge of the lid and left the vessel in the centre. To avoid disturbing the measurements of ammonia emission, a total of 32 vessels was used, two vessels for each pig: one for measuring ammonia emission and the other for pH. Ammonia in the outgoing air was removed by passing the air flow through two impingers, each containing 70 mL of 1 M HNO<sub>3</sub>. The second impinger served as a control and contained not more than 5% of the ammonia trapped in the first impinger. The air left the system after passing a water trap, a flow controller adjusted to 4.2 L/min and a pump. The first impinger was replaced daily and the second impinger was replaced after 7 days. From the first and second impingers both the ammonia concentration and the volume of the liquid were determined. The daily ammonia emission was calculated by multiplying the volume with the ammonia concentration. At the end of the in vitro ammonia emission measurement, the slurry was sampled for chemical analyses. The pH of the slurry was measured daily, in the parallel vessel.

#### Chemical analyses

All samples were analysed in duplicate. The slurry was analysed for DM, ash, N, and CF according to AOAC (1990) procedures, and  $NH_4^+$  was determined spectrophotometrically according to NEN 6472 (NEN, 1983). Volatile fatty acids were measured on a Packard 427 gas chromatograph, equipped with a flame ionisation detector (Derikx *et al.*, 1994). The method used for carbonates was essentially the same as that described by Amundson *et al.* (1988) using ethane as an internal standard. The pH was measured at room temperature with a Hanna instrument glass electrode (model HI 8417) directly submerged in the slurry. Data on nutrient composition are from CVB (1994) in which data on chemical composition, digestibility, and feeding value of feefstuffs have been presented along with the references for determination of chemical composition.

### Statistical analysis

The individual pig was the experimental unit. The effect of the diet (grains, by-product, tapioca and sugar beet pulp) on the slurry composition was analysed by one-way ANOVA using the GENSTAT statistical package. When the *F*-test showed a significant effect of diet (P < 0.05), means were separated with the LSD procedure with a confidence level of 0.05 (Genstat 5 committee, 1993). The effect of diet on the pH and the ammonia emission was tested within and between days by using the analysis of variance for a repeated measurement (Genstat 5 committee, 1995). The effect of slurry composition on the pH of slurry was tested, using one-way regression analyses (Genstat 5 committee, 1993).

## Results

#### Ammonia emission and pH

The effects of the diet on the ammonia emission from slurry over 7 days are illustrated in Figure 1. The overall mean of ammonia emission during the 7-day measuring period was 28.60 mmol/d. The mean ammonia emission over the 7-day measuring period was strongly affected by diet (P < 0.001). The mean ammonia emission was similar for the grain, the by-product and the tapioca based diets (averaged 31.3 mmol/d), but was much lower (17.23 mmol/d) for the sugar-beet pulp based diet. In general, during the measuring period, ammonia emission increased (P < 0.05) with time and reached a plateau after 2

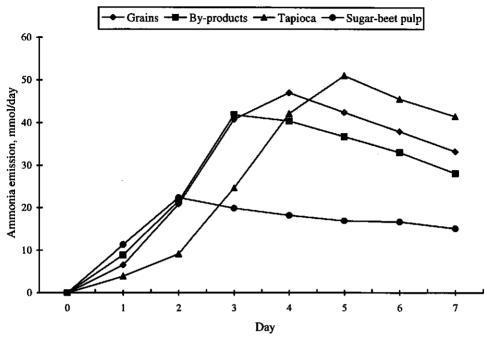


Figure 1. Ammonia emission from slurry of pigs fed different diets during 7 days of the in vitro measurement

to 4 days (P < 0.05). Differences between diets varied during the 7-day measuring period. During day 1, the ammonia emission was highest for the sugar-beet pulp based diet. During day 2 and day 3, the mean ammonia emissions showed large variations. From day 4 onwards, the slurry of the sugar-beet pulp based diet had the lowest ammonia emission (P < 0.001).

Total nitrogen losses during the 7-day measuring period, calculated, as a percentage of the daily nitrogen excretion (in urine and faeces) was different between diets (P < 0.001). Nitrogen losses were 23.69, 23.76 and 21.59% for the grain, the by-product and the tapioca based diets, respectively. Total nitrogen losses from slurry of pigs fed the sugar-beet pulp based diet were 14.03%, approximately 38% lower than the other three diets.

The pattern of the slurry pH with time paralleled the pattern in ammonia emission with time (Figure 2). For the grain, the by-product and the tapioca based diets, the pH of the slurry increased during the first 3 days and then reached a plateau. The pH of the slurry from pigs fed the sugar-beet pulp based diets tended to decrease just one day after the start of the ammonia emission measurements. The mean pH over the 7-day measuring period of slurry from pigs fed the grain, the by-product and the tapioca based diets were 8.9, 8.80 and 8.83, respectively. Differences between these diets were not significant (P > 0.05). The mean pH of slurry from the pigs fed the sugar-beet pulp based diet was 8.07, approximately 0.8 to 0.9 unit lower than the other three diets (P < 0.001).

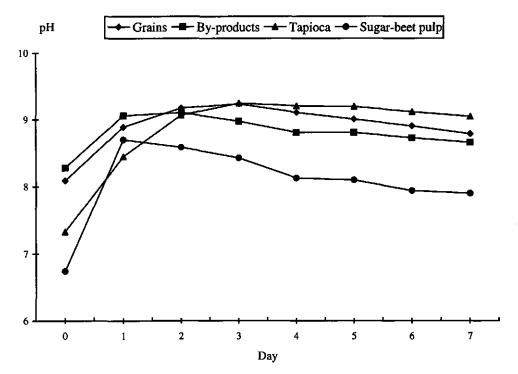


Figure 2. The pH of slurry measured during 7 days of the in vitro measurement of ammonia emission from slurry of pigs fed different diets

#### Composition of the slurry

The chemical composition of the slurry from pigs fed different diets is shown in Table 3. In general, the chemical composition of the slurry of pigs on day 7 was more influenced by diet than on day 0. The DM content of the slurry on day 0 was not different between diets (P > 0.05). On day 7, it was lowest in the slurry of the tapioca based diet (P < 0.01). The ammonium concentration in slurry of pigs fed the sugar-beet pulp based diet was highest (P < 0.05) on day 0, but was lowest on day 7 (P < 0.001). There was no difference in ammonium concentration of the slurry of pigs fed the by-product based diet on both day 0 and day 7. On day 0, the carbonate concentration was lowest in the slurry of pigs fed the sugar based diet (P < 0.001). The diet strongly affected the total VFA concentration in the slurry during the 7-day measuring period (P < 0.001). The total VFA concentration of the slurry after 7 days was higher in the slurry of pigs fed the

		D	iet			
Variable	Grains (4) <sup>b</sup>	By-products (4)	Tapioca (4)	Sugar beet pulp (4)	<sup>-</sup> P <sup>a</sup>	SEM
DM, g/kg			<sup>"</sup> "			
D 0	73.4	76.5	65.5	73.4	NS	4.14
D 7	75.3°	84.8 <sup>c</sup>	64.6 <sup>d</sup>	77.2°	**	3.57
D 0 – d 7	2.0	8.2	-0.9	2.5	NS	2.76
NH4- N, g/kg						
D 0	0.56°	0.56°	0.64°	1.13 <sup>d</sup>	*	0.11
D 7	4.23°	3.48 <sup>d</sup>	4.75°	3.24d	***	0.19
D 0 – d 7	3.66 <sup>cd</sup>	2.92 <sup>cf</sup>	4.11 <sup>d</sup>	2.61 <sup>ef</sup>	*	0.30
Total N, g/kg						
D 0	7.04°	6.34°	7.37 <sup>d</sup>	6.22°	*	0.27
D 7	6.21 <sup>cd</sup>	5.59°	6.74 <sup>d</sup>	6.15 <sup>cd</sup>	**	0.23
D 0 – d 7	-0.82	-0.75	-0.63	-0.29	NS	0.18
Ash, g/kg						
D0	16.6°	24.1 <sup>d</sup>	17.6°	15.6 <sup>c</sup>	***	1.12
D 7	19.0 <sup>°</sup>	29.1 <sup>d</sup>	20.4 <sup>e</sup>	17.3°	***	0.49
D 0 – d 7	2.63 <sup>cf</sup>	2.75°	4.55 <sup>d</sup>	1.48 <sup>ef</sup>	***	0.28
Carbonate, L/kg						
D 0	1.56°	2.19 <sup>c</sup>	0.99 <sup>d</sup>	1.71c	*	0.27
D 7	4.55°	2.91°	4.43°	2.72 <sup>d</sup>	***	0.15
D 0 – d 7	3.00 <sup>c</sup>	2.72 <sup>cf</sup>	3.44°	1.6 <sup>df</sup>	*	0.41
Total VFA, g/kg						
D0	2.99	2.41	1.14	4.21	NS	0.91
D7	4.77°	6.58 <sup>d</sup>	3.78°	7.47 <sup>d</sup>	***	0.50
D 0 - d 7	2.49	4.17	2.64	3.31	NS	0.59

Table 3. Chemical composition of slurry at day 0 and day 7 from pigs fed different diets

<sup>a</sup>Probability of a significant treatment effect. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; NS not significant <sup>b</sup>Number of observation in parentheses.

<sup>c,d,e,f</sup>Different letters in superscript indicate significant difference (P < 0.05).

sugar-beet pulp based (7.47 g/kg) and the by-product based diets (6.58 g/kg) than in the grain based (4.77 g/kg) and the tapioca based (3.78 g/kg) diets.

The relationship between the chemical composition of the slurry on day 0 and day 7 and the slurry pH at these days was assessed. In Table 4, one-way regression coefficients of the slurry pH on the major chemical components were calculated. On day 0, the pH was negatively related to the  $NH_4^+$  content (P < .05;  $R^2 = 0.23$ ). Other components, including DM, ash, carbonate and total VFA did not significantly relate to the pH of the slurry.

• · · · · · ·	Day (	)		Day	7	
Variable	ß <sup>a</sup> ± SE	P <sup>5</sup>	R <sup>2</sup> (%)	<u> </u>	P°	R <sup>2</sup> (%)
NH₄-N, g/kg	-0.00087 ± 0.00038	*	0.23	0.00054 ± 0.00014	**	0.49
DM, g/kg	$0.007 \pm 0.0158$	NS		$-0.018 \pm 0.015$	NS	
Ash, g/kg	$0.023 \pm 0.03$	NS		$0.048 \pm 0.027$	NS	
Carbonate, L/kg	-0.18 ± 0.20	NS		$0.52 \pm 0.11$	***	0.60
Total VFA, g/kg	$-0.0001 \pm 0.0001$	NS		$-0.00025 \pm 0.00006$	***	0.55

 Table 4. One-way regression coefficients of the slurry pH on the chemical composition of the slurry on days 0 and 7

<sup>a</sup>Regression coefficient

<sup>b</sup>Probability of a significant treatment effect. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; NS not significant

On day 7, the pH was strongly and positively related to the  $NH_4^+$  ( $R^2 = 0.49$ ) and carbonate ( $R^2 = 0.60$ ) contents. The total VFA content negatively related to the pH ( $R^2 = 0.55$ ). Ash and DM contents did not relate to the pH of the slurry on day 7.

To quantify the effect of the pH and the logarithm of  $NH_4^+$ -N concentration on day 7 on the logarithm of ammonia emission, the following regression equation was calculated:

Log ammonia emission = 
$$-4.21 + 0.45 (\pm 0.13) \times (pH) + 0.44 (\pm 0.30) \times \log (NH_4-N);$$
  
 $R^2 = 0.67$ 

The ammonia emission was strongly affected by the pH of the slurry. For each unit decrease of the slurry pH, the ammonia emission decreased by 45%.

## Discussion

The main objective of this research was to evaluate the feasibility of reducing ammonia emission from slurry of growing-finishing pigs by dietary composition. In this study, the diets were similar in NE and CP contents and differed mainly in NSP content and dEB. It was observed that the ammonia emission was lowest for the sugar-beet pulp based diet, which had the highest content of dietary NSP and the lowest dEB. According to Muck and Steenhuis (1981), the main part of ammonia emission originates from urea in urine. Urea is converted into ammonia and carbon dioxide by urease present in faeces. As soon as the urine comes into contact with faeces, the conversion starts (Stevens *et al.*, 1989; Aarnink, 1997). In the slurry, the equilibrium vapour pressure of NH<sub>3</sub> is controlled by the NH<sub>3</sub> concentration in the solution, which is mainly affected by the ammonium concentration, temperature and the pH of the slurry (Muck and Steenhuis, 1981). In the present study, at a fixed temperature, the ammonium content and the pH of slurry were the main factors influencing the ammonia emission.

(1)

The ammonium content of the slurry on day 0 and day 7 was significantly affected by the diet. The dietary levels of NSP and dEB are important in that respect. Dietary non-starch polysaccharides and dEB influenced the ammonium concentration of slurry in different ways. The dEB level mainly affected the renal excretion of urinary ammonium. In the present study, dEB between diets differed by adjusting the dietary Na, K and Cl levels. According to Tucker *et al.* (1988), any alteration in the relative amount of Na, K and Cl in the diet will affect the urinary excretion of H<sup>+</sup> and NH<sub>4</sub><sup>+</sup>. When dEB decreases, in order to maintain the acid-base status of the body, the animal increases the net acid excretion. The pH of urine decreases. The increase of renal H<sup>+</sup> excretion is accompanied by an increase in NH<sub>4</sub><sup>+</sup> excretion.

We have recently published detailed data on the compositions of urine and faeces, where we showed that the ammonium content of faeces was not affected by the diets, whereas, the ammonium content of urine was highest in the urine from pigs fed the sugar-beet pulp based diet (Canh *et al.*, 1997). Therefore, the relatively high content of slurry ammonium of pigs fed the sugar-beet pulp based diet on day 0, when urinary urea was not converted into slurry ammonium yet, was caused by the high content of urinary ammonium. However, the pattern of ammonium in the slurry changed differently during the 7-day period of slurry storage. After 7 day of storage, the ammonium content of the slurry was almost entirely determined by the urea content of the urine before mixing to slurry (on day 0). According to Canh *et al.* (1997) differences in the urea content of the urine were caused by differences in the amount of NSP in the diets. Nitrogen excretion is shifted from urea in urine to bacterial protein in faeces when more fibrous feedstuffs are included in the diet (Kirchgessner *et al.*, 1993; Canh *et al.*, 1997). The increasing dietary level NSP in the sugar-beet pulp based diet reduced the urea content in urine and in the slurry. This, consequently, reduced the ammonium content, thereby, reducing the ammonia emission.

As expected, the ammonia emission was significantly influenced by the pH of the slurry. The calculated regression coefficient (on day 7) in this research was 0.45 (Equation 1). According to Sommer and Husted (1995), the slurry pH is of great importance for the government of ammonia volatilisation from pig slurry. Because the effect of pH on ammonia volatilisation is very strong, a minor change in pH can have a large effect. In this study, the pH of slurry was lowest in pigs fed a high-NSP and low-dEB diet. This can be explained by three main reasons: Firstly, a low dEB in the diet reduces the pH of the urine. Secondly, a high level of dietary NSP enhances the microbial activities in the hind gut of pigs increasing VFA formation in faeces and in the slurry during storage. Thirdly, a high-NSP diet causes a shift of N from urine to faeces causing a reduction in the ammonium content of the slurry.

In general, the pH of slurry is mainly affected by its contents of ammonium, VFA and carbonates (Sommer and Husted, 1995). During the 7-day period of ammonia emission measurements, ammonium was lower and VFA was higher in slurry of pigs fed the sugar-beet pulp based diets than for the other three diets. Volatile fatty acids are mainly produced from faeces by anaerobic microbial fermentation of undigested dietary fibber and by deamination of amino acids (Imoto and Namioca, 1978; Farnworth *et al.*, 1995). Spoelstra (1979) reported that during the storage of slurry, numerous organic compounds of low molecular weight are accumulated. Among these, the VFA are quantitatively the most important group.

The decomposition of fermentable carbohydrates is accompanied by the formation of  $CO_2$ . At the same time bacteria require  $CO_2$  to synthesise acetic acids and amino acids (Allison, 1969; Imoto and Namioca, 1978; Spoelstra, 1979). Thus, the increased microbial activities in the slurry of pigs fed the sugar-beet pulp based diet together with a low CaCO<sub>3</sub> content of this diet may explain the low carbonate content in the slurry of this diet on day 7. Because the urea in the urine is converted into ammonium and carbonate, the low urea concentration in the urine of pigs fed the sugar beet pulp-based diet may also have contributed to the low carbonate content in the slurry of this diet.

The pH of the slurry on day 7 seemed to be related more with ammonium, carbonate and total VFA than that on day 0. The calculated coefficient for the pH on the major chemical compositions of the slurry on day 0 and day 7 demonstrated that the pH of the slurry seemed to be affected by a combination of different factors. On day 0, the low regression coefficient ( $R^2 = 0.23$ ) between the pH and the ammonium contents indicated that the ammonium content was not the sole factor affecting the pH of the slurry. The pH of the slurry on day 0 was measured directly after mixing the urine and faeces. It could be influenced by the large interaction between different components of the slurry. On day 7, the pH of slurry was significantly influenced by ammonium, total VFA and carbonate contents. It was found that the ammonium content had a lower correlation coefficient with the pH than the other two factors. This might be due to the fact that a significant amount of ammonia evaporated during the ammonia emission measurement. The slurry with a higher pH value lost more ammonia than the slurry with a lower pH value. Consequently, differences in the ammonium content between diets became smaller after 7 day of the ammonia emission measurement.

In this research, the ammonia emission was measured for a short time with an in vitro laboratory system. In commercial pig houses, the situation is different, mainly because of the dynamic situation in which continuously fresh urine and faeces are added to the slurry pit. Ad libitum water gift in commercial pig houses may result in differences in water intakes and thereby may influence the ammonia emission from slurry. Also in this research dEB and NSP were not altered independently among the four dietary treatments. Further research is necessary to describe the independent effect of dEB and NSP on slurry characteristics. Nevertheless, the potential impact of slurry characteristics derived from different diets on ammonia emission has been demonstrated in this research.

## Conclusion

There are possibilities to reduce the pH of slurry by the dietary composition. Feeding diets with a low dietary electrolyte balance and a high content of non-starch polysaccharides, like the sugar-beet pulp based diet, increases the volatile fatty acid concentration in the slurry, reduces the pH and the ammonia emission from the slurry of growing-finishing pigs. This approach may be an economic way to reduce ammonia emission from pig farming.

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## **Chapter 4**

# Dietary Carbohydrates alter Faecal Composition and pH and Ammonia Emission from Slurry of Growing Pigs

## T. T. Canh<sup>a, b, c, \*</sup>, A. L. Sutton<sup>d</sup>, A.J.A. Aarnink<sup>a</sup>, M.W.A. Verstegen<sup>b</sup>, J. W. Schrama<sup>b</sup> and G.C.M. Bakker<sup>e</sup>

<sup>a</sup>Department of Livestock Engineering, Institute of Agricultural and Environmental Engineering (IMAG-DLO), Wageningen, The Netherlands.

<sup>b</sup>Wageningen Institute of Animal Science (WIAS), Departments of Animal Nutrition and Animal Husbandry, Wageningen Agricultural University, The Netherlands.

°Netherlands Foundation for the Advancement of Tropical Research.

<sup>d</sup>Department of Animal Sciences, Purdue University, USA.

\*Department of Nutrition of Pigs and Poultry, Institute of Animal Science and Health (ID-DLO), Lelystad, The Netherlands.

\*Tel. (31) 317 476552; Fax: (31) 317 425670; E-mail: T.T.Canh@IMAG.DLO.NL

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T. T. Canh, A. L. Sutton, A.J.A. Aarnink, M.W.A. Verstegen, J. W. Schrama and G.C.M. Bakker

## Abstract

We investigated the effect of dietary carbohydrates on composition and pH of faecal material and ammonia emission from the slurry of growing pigs. Thirty-four barrows (about 40 kg BW) were randomly allotted to one of 10 diets. A basal diet was formulated to meet all requirements for protein, amino acids, minerals and vitamins. The control diet was composed of the basal diet plus heat-treated cornstarch. In the other diets, cornstarch in the control diet was replaced with three levels of either coconut expeller, soybean hulls or dried sugar beet pulp. Faeces were collected separately from urine in a balance experiment. Faeces were mixed with a standardized urine (ratio of 1:2.5, wt/wt) to form a slurry. A sample of this slurry was placed in an in vitro system to determine the pH and ammonia emission for 16 days at 20° C. The faecal and slurry DM contents decreased (P < 0.001) and the total VFA concentrations increased (P < 0.001) when the level of dietary carbohydrates increased. The pH and ammonia emission decreased as the level of carbohydrates increased (P < 0.001). Addition of soybean hulls to the diet had the greatest effect on reducing the pH and ammonia emission (P < 0.001), while the effect of sugar beet pulp and coconut expeller were about the same. A linear relationship was found between the intake of dietary non-starch polysaccharides (NSP) and the ammonia emission (P <0.001). For each 100 g increase in the intake of dietary NSP, the slurry pH decreased by about 0.12 unit and the ammonia emission from slurry decreased by 5.4%. We conclude that replacing cornstarch in the diet by components with a high concentration of fermentable carbohydrates increases the VFA concentration of faeces and slurry, and reduces the pH and ammonia emission from the slurry of growing pigs.

### Key Words: Pigs, Carbohydrates, Fermentation, pH, Ammonia

## Introduction

Ammonia emissions from livestock production systems cause environmental pollution and therefore should be reduced (Apsimon and Kruse-Plass, 1990). A major source of ammonia emission

is urea excreted via urine. Urea is converted into ammonia and carbon dioxide by the urease present in facces. The most important factors affecting this process are the urinary urea concentration and the pH and temperature of slurry (Muck and Steenhuis, 1981; Stevens et al., 1989; Sommer and Husted, 1995). One approach to reduce the ammonia emission is through diet manipulation (Gatel and Grosjean, 1992; Heinrichs and Oldenburg, 1993; Kreuzer and Machmüller, 1993). In pigs, there is clear evidence that dietary fibre can affect the partitioning of nitrogen excretion between urine and faeces and reduce urinary urea excretion (Mroz et al., 1993; Schulze et al., 1995; Canh et al., 1997a). Canh et al. (1997a, 1997b) demonstrated that the inclusion of 30% sugar beet pulp in a pig diet lowered the slurry pH by 0.97 unit and the ammonia emission from slurry by 47%. The latter authors concluded that the slurry pH was related to slurry VFA concentrations. In pigs, VFA are mainly produced by microbial fermentation of dietary fibre in the large intestine (Imoto and Namioka, 1978; Salvador et al., 1993) and during slurry storage (Spoelstra, 1979; Canh et al., 1997b). Although much is known about the relationship between dietary fermentable carbohydrates and VFA production in pigs, little research has been done to qualify the relationship between dietary fermentable carbohydrates and faecal characteristics and the ammonia emission. Therefore, the objective of this study was to investigate the influence of the level and source of dietary carbohydrates on the faecal composition and the resulting pH and ammonia emission from slurry of growing pigs.

## **Materials and Methods**

## Animals and Diets

A total of 34 castrates [Large White  $\times$  (Dutch Landrace  $\times$  Finnish Landrace)] were allotted to a control diet and nine experimental diets arranged as a 3  $\times$  3 factorial with three sources of carbohydrates and three levels of each carbohydrate source. Each kilogram of the control diet contained 340 g basal diet and 660 g heat treated cornstarch. The basal diet was formulated (Table 1) to meet the required amounts of protein, amino acids, minerals and vitamins (ARC, 1981) in the control diet. The cornstarch in the control diet was replaced with three levels each by equivalent amount of DE of 134 g cornstarch of coconut expeller (C1, C2, C3), soybean hulls (S1, S2, S3), or dried sugar beet pulp (B1, B2, B3) (Table 2). The chemical composition of the experimental diets is shown in Table 3. Diets differed in level and composition of non-starch polysaccharides (NSP). The soybean hulls based diets had the highest level of fermentable portions (cellulose and hemicellulose) of NSP, followed by diets based on sugar beet pulp and the coconut expeller. The coconut expeller based diets contained more lignin than the other two sources. These NSP-sources also contained crude protein, which increased the dietary protein levels. However, because of their relative poor quality (CVB, 1997) the basal diet was not formulated for each treatment. In addition, this design allowed the interpretations of the practical consequences for formulating pig feeds.

Ingredients	Amount, g/kg
Alfalfa meal	391.0
Potato protein, dried	426.1
Soybean oil	28.6
Cane molasses	100.0
CaHPO4	26.0
Limestone	13.1
Salt	7.1
Premix (without antibiotics	s) <sup>a</sup> 4.0
DL-Methionine	2.3
Choline chloride	0.9
L-Tryptophan	0.9

Table 1. Ingredient con	position of	' the basa	l diet
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<sup>a</sup>The vitamin and mineral premix supplied per 1 kg of the basal diet: 22860 IU of vitamin A, 4570 IU of vitamin D<sub>3</sub>, 23 mg of vitamin E, 11 mg of riboflavin, 22 mg of d-pantothenic acid, 57 mg of niacin, 57  $\mu$ g of vitamin B<sub>12</sub>, 0.1 mg of biotin, 0.5 mg of Co; 91.2 mg of Se, 4.3 mg of I, 246.9 mg of Fe, 45.5 mg of Cu, 110.6 mg of Mn, 161.2 mg of Zn, 357 mg of antioxidant.

The pigs were housed in groups of four each, and were individually fed their respective diets from 40 kg BW until 55 kg BW. When the pigs reached 55 kg BW, they were individually housed in an environmentally controlled room in metabolism cages that allowed the separate collection of urine and faeces. Four pigs were used for each of seven diets (control, C2, C3, S1, S2, B1, B3). Because of a technical problem before the start of the experiment, only two pigs were used for the other three diets (C1, S3, B2). The room temperature was maintained at about 20°C. The pigs were fed two times the energy requirement for maintenance, which was assumed to be 435 kJ DE per kg BW<sup>0.75</sup> (ARC, 1981). The diets were mixed with water 8 h before feeding (0.263 L per kg BW<sup>.75</sup>) and fed twice daily. No additional water was offered to the pigs.

#### Faeces and Urine Collection

The 22-day experimental period consisted of a 10-day adaptation period to allow the pigs to become accustomed to the cages and a 12-day period during which urine and faeces were collected. The faeces from each pig collected during the first 2 days of the collection period were stored at 4°C until they were used to make slurry at the end of the 12-day collection period for ammonia emission measurement. Faeces collected during the last 10 days of the collection period were stored at  $-20^{\circ}$ C. After this 10-day period, faeces of two pigs within the same diet were pooled and sampled for chemical analysis.

#### Chapter 4

Compositions,	Control	Coc	onut exp	eller	S	oybean h	ulls	Dried	l sugar b	eet pulp
(g as-fed basis)		C1	C2	C3	S1	<b>S</b> 2	<b>S</b> 3	B1	B2	B3
Basal diet	340	340	340	340	340	340	340	340	340	340
Corn starch	660	526	392	258	526	392	258	526	392	258
Coconut expeller		156	312	468						
Soybean hulls					192	384	576			
Dried sugar beet pulp								184	368	552
Cornstarch	0	20	40	60	20	40	60	20	40	60
Replaced (%)										

Table 2. Formulation of the experimental diets

Because it was the objective of this experiment to determine the effect of dietary fermentable carbohydrates on the faecal composition and, subsequently, on the pH and ammonia emission from slurry, a standard urine was used to make the slurry. This was done to prevent confounding effects of ammonium concentration in urine. Urine, collected from all pigs on all diets during the collection period, was pooled, mixed and neutralised to a pH of 8 (the standard urine) and stored at -20°C. Previous research in our laboratory has shown that simply mixing fresh urine and faeces produced a more alkaline slurry (about 0.3 unit) than normally found under practical conditions (Canh, 1994), which may influence the concentration and/or species of microorganisms in the slurry.

Compositions,	Control	Coco	nut expe	ller	Soybe	an hulls		Dried s	ugar be	et pulp
(g as-fed basis)		C1	C2	C3	<b>S</b> 1	S2	<b>S</b> 3	B1	<b>B</b> 2	B3
DM	930	923	915	908	922	915	909	924	920	913
Ash	930 37	923 46	54	63	922 44	51	909 57	724 48	56	69
CP	151	182	212	241	167	181	195	161	169	181
Crude fat	20	34	46	59	27	33	38	22	22	24
NSP <sup>a</sup>	153	217	282	341	270	375	472	262	335	452
Cellulose	48	68	88	107	110	166	216	78	98	130
NDF	68	128	185	240	170	261	343	139	188	264
ADF	50	84	117	149	124	191	251	84	107	143
ADL	14	22	29	37	16	18	19	15	16	18
Starch	620	484	352	226	470	335	213	472	373	212
Sugar	19	37	54	70	22	25	28	35	45	62
Hemicellulose	36	44	68	91	46	70	92	55	81	121
GE	18.36	18.63	18.88	19.13	18.46	18.53	18.61	18.2	2 18.1	2 18.00

Table 3. Analyzed chemical composition of the experimental diets (in g/kg DM except DM in g/kg feed and GE in MJ/kg DM)

<sup>a</sup>Non-starch polysaccharides, determined as: organic matter – (crude protein + crude lipid + starch + sugar).

The urine was neutralised by converting urea into ammonia with an addition of 0.1% (wt/wt; wet weight basis) of faeces, sampled from the control diet during the collection period, to the urine. After mixing for 10 min and incubating for 24 h, the urine was acidified to a pH of 8 with hydrochloric acid and stored until the ammonia emission measurement was performed. The NH<sub>4</sub>-N, total-N, DM, and ash contents of the standard urine were 2.86, 3.17, 9.0 and 4.03 g/kg, respectively.

Faeces collected from each pig were mixed with the standard urine in a ratio of 1:2.5 (wt/wt; wet weight basis) to make the slurry. A sample of this slurry was placed in an in vitro system located in a 20°C temperature controlled room for measuring ammonia emission.

### Ammonia Emission

The ammonia emission was determined in vitro for a period of 16 days according to the procedures described by Derikx and Aarnink (1993). Slurry (2 kg) was placed in a 6.5-L vessel covered by a lid. The surface area of the slurry in the vessel was 284 cm<sup>2</sup>. Air entered the vessel through small holes at the edge of the lid and left the vessel through the centre. Ammonia in the outgoing air was removed by passing the airflow through two impingers, each containing 70 mL of 1 M HNO<sub>3</sub>. The second impinger served as a control and contained no more than 5 % of the ammonia trapped in the first impinger. The air exited the system after passing a water trap, an air- flow controller adjusted to 4.2 L/min and a pump. The first impinger was replaced after 1, 2, 4, 8 and 16 days of the measuring period. The second impinger was replaced after 8 and 16 days. Ammonia concentration and the volume of the liquid were determined in both impingers. From the first and the second impingers, the ammonia emission was calculated by multiplying the volume with the ammonia concentration. The cumulative ammonia emissions were measured from the start to day 1, from day 1 to day 2, from day 2 to day 4, from day 4 to day 8 and from day 8 to day 16 and the total accumulated ammonia emission over the 16-day measuring period per kg of slurry was determined. The pH of slurry was measured at the same time when the first impinger was replaced.

## **Chemical Analyses**

Faeces were analysed for DM, ash, N, crude fat, crude fibre and total VFA according to AOAC (1990) procedures and  $NH_4^+$  was determined spectrophotometrically according to NEN (1990) procedures. Volatile fatty acids were measured on a Packard 427 gas chromatograph (Hewlett Packard, model 427, Downers Grove, IL), equipped with a flame ionisation detector (Derikx et al., 1994). The pH measurements were performed at room temperature (20°C) with a glass electrode (Hanna Instruments, glass electrode, model HI 8417, Limena, Italy) directly submerged in the slurry. The pH was measured at the top layer (about 1 cm beneath the surface), middle layer (about 6 cm beneath the surface) and bottom layer (about 12 cm beneath the surface) of the slurry column. Weighted averages of pH of the

upper, middle and bottom layers of the slurry were calculated for statistical analyses using the average pH for the number of days between two successive pH measurements. At the end of the emission measurements, the slurry was mixed and sampled for chemical analyses. The samples obtained from each diet were analysed in duplicate for DM, CP, ash, NDF, ADF, ADL, cellulose, hemicellulose, starch, sugar and energy, following Dutch protocols (NEN, 1990), most of which are similar to those of the Association of Official Analytical Chemists (AOAC, 1990). Dietary NSP was determined as OM - (CP + crude lipid + starch + sugar).

#### Statistical Analyses

The effect of source and level of fermentable carbohydrates on the chemical composition of faeces, slurry, and the pH and ammonia emission from the slurry was analysed by ANOVA using the GENSTAT statistical Package (Genstat 5 committee, 1993) with the following model:

$$Y_{ijk} = \mu + S_i + L_j + (S \times L)_{ij} + e_{ijk} \tag{1}$$

Where  $Y_{ijk}$  = dependent variable,  $\mu$  is the overall mean;  $S_i$  = the effect of source of fermentable carbohydrates (i = coconut expeller, soybean hulls and sugar beet pulp);  $L_j$  = level of carbohydrates (J = 1..3);  $S \times L$  = interaction;  $e_{ijk}$  is the residual error. For the chemical composition of faeces, the standard errors of the means were calculated only from the treatments with replicates. The LSD procedures were used to compare treatment means. The effect of the intake of dietary NSP on the total VFA concentration of faeces and slurry, and on the average pH of slurry and the ammonia emission was tested, using regression analyses. These analyses were done in two steps. In the first step, the interaction effect between NSP intake and source of NSP was included in the model (without the control diet). Because the interaction effect was not significant, in the second step, this interaction was left out of the model and the control diet was included in the analysis.

## Results

The effect of dietary fermentable carbohydrates on production and chemical composition of the faeces is shown in Table 4. Both the level (P < 0.001) and source (P < 0.001) of fermentable carbohydrates used to replace cornstarch in the control diet influenced the quantity of faeces produced by the pigs. Diets containing soybean hulls gave the highest faeces production (P < 0.05) followed by diets with dried sugar beet pulp and coconut expeller, respectively. Faeces production of all diets was negatively related to the faecal DM content (P < 0.01).

		ပိ	Coconut expeller	aller	Soy	Soybean hulls	S	Uneu s	Dried sugar beet pulp		•	
		G	ß	ទ	SI	S2	S3	B1	B2	B3	<b>h</b> ,	SEM
Amount, g/d	310	410		781	638	954	1481	560	869		S‴,L,S×L).	34
DM. e/kg	402	379		328	325	300	286		290		S".L	
Ash, g/kg	51.1	53.4	48.3	45.7	38.4	34.3	29.2	54.2	56.6	51.2	S‴,L	2.32
Total-N, g/kg	12.8	13.7		14.7	11.8	11.3	9.5		11.5		s	0.41
NH4-N, g/kg	1.03	0.90		2.05	0.80	2.13	2.65		1.85		S <sup>*</sup> ,L <sup>**</sup> ,(S×L)	0.17
Crude fiber, g/kg	141			86	108	100	112		68		ŝ	
Crude fat, g/kg	27.5			20.4	23.7	21.5	16.8		22.1		<b>ٿ</b>	
Total VFA, g/kg	3.70			5.14	5.52	5.71	6.32		5.33		s",L"	0.23
Compositions	Control	Cocoi	Coconut expeller			Soybean hulls	- 1	Dried si	Dried sugar beet pulp	pulp	2	4
		CI	ບ ເ	ប៊	SI	<b>S</b> 2	S3	Bl	B2	B3	Þ.	SEM
pH												
Upper	8.3	4.8	8.2	8.2	8.0	8.0	7.3	8.2	8.2	8.1	S,L, (SxL)	0.08
Middle	8.0	7.6	<i>T.T</i>	7.4	7.4	7.4	6.6	7.6	<i>L.L</i>	7.5	s",L"	0.15
Bottom	7.4	7.2	7.1	7.0	6.9	6.8	6.3	7.1	6.9	7.1	°,	0.15
Average	7.9	7.7	LL	7.6	7.4	7.4	6.7	7.6	7.5	7.5	s",L	0.11
Ammonia	2.96	2.68	2.42	2.37	2.46	2.35	1.90	2.77	2.48	2.26	s",L",(S×L)	0.05
emission												

Therefore, a larger faeces weight was partly caused by higher water content. However, also larger amounts of faecal DM were excreted with increasing levels of fermentable carbohydrates in the diets. Ammonium and total VFA concentrations of faeces, increased (P < 0.001) when the level of fermentable carbohydrates increased (Table 4). There was an effect of the interaction between the level and source of carbohydrates on faecal ammonium concentration. The addition of soybean hulls to the diet resulted in the highest total VFA concentrations in faeces (P < 0.01), followed by the dried sugar beet pulp and the coconut expeller diets (P < 0.10). The faecal ash concentrations from pigs fed the coconut expeller and the sugar beet pulp diets were similar to those of the control diet (P > 0.05). The faecal ash contents obtained from pigs fed the soybean hulls diets were lower compared with the other three diets (P < 0.001) and similar for the soybean hulls and the dried sugar beet pulp diets (P > 0.05). In general, increased levels of dietary fermentable carbohydrates lowered the crude fat and crude fibre (P < 0.001) contents in faeces. However, this was not the case for pigs fed soybean hulls diet. Different sources of fermentable carbohydrates did not influence the crude fat content of faeces.

In Table 5, the mean pH of the slurry measured during the 16-day in vitro measuring period from the different levels and their averaged pH is shown. With the exception of the pH measured at the bottom layer of the slurry (P = 0.24), both the source (P < 0.001) and the level (P = 0.012) of fermentable carbohydrates strongly affected the pH of the slurry. In general, slurry pH was lower at the higher levels of fermentable carbohydrates included in the diet (P < 0.05). The addition of soybean hulls to the diet gave the strongest pH reduction. The pH of slurry from pigs fed dried sugar beet pulp diet was slightly lower than that observed with the coconut expeller diet. The pH in the middle level of the slurry was very similar to the average pH of the three levels. The pH of the upper layer was related to the pH of the middle and bottom levels as follows:

$$Upper-pH = 3.55 (SE = 0.58) + 0.61 (SE = 0.08) \times middle-pH; \qquad (R^2 = 0.65)$$
(2)

$$Upper-pH = 4.08 (SE = 0.77) + 0.57 (SE = 0.11) \times bottom-pH; \qquad (R^2 = 0.45)$$
(3)

The cumulative ammonia emission from the slurry is shown in Table 5. The source and level of fermentable carbohydrates in the diet strongly affected the ammonia emission. Increasing the level of fermentable carbohydrates in the diet reduced the ammonia emission. For each per cent of cornstarch replaced with coconut expeller, soybean hulls and dried sugar beet pulp, the ammonia emission from slurry decreased by 0.35, 0.51 and 0.36%, respectively. The slurry from pigs fed the soybean hulls diet gave the lowest ammonia emission (P < 0.001). Ammonia emission was similar for the coconut expeller and the dried sugar beet pulp diets (P > 0.05). The diet based on cornstarch (the control) had the highest slurry ammonia emission (P < 0.001).

				Ì	Î							
	Control	Coc	nut expel	ler	So	ybean hull	s	Dried 5	sugar bee	t pulp		
Compositions		บิ	3	ទ	SI	S2	S3	B1	B2	B3	Å	SEM
	5	121			011	2	٤			ŲĘ.	*** 1 *** 1	V F V
LIM, B/KS	[4]	+C1			117	<b>1</b>	77	120		5	٥ ٢	4.14
Ash, g/kg	24.3	26.8			93	18.4	14.2	24.9		23.9	S",L"	1.01
Total-N, g/kg	6.5	7.3	6.8	7.2	6.1	6.3	4.8	6.2	5.7	5,4	S",L",(S×L)	0.26
NH4-N, g/kg	2.25	2.25			2.03	2.40	2.03	2.25		2.20		0.12
Total VFA, g/kg	4.32	4.11			5.52	6.72	7.24	5.18		5.96	s""L"	0.39

Table 6. Composition (on fresh basis) of slurry after the in vitro ammonia emission experiment

<sup>a</sup>Significant level of treatment factor: S, L: source and level of fermentable carbohydrates,: (S×L): interaction, P < 0.05; P < 0.01; P < 0.001. <sup>b</sup>Standard error of mean.

Table 7. Regression coefficients of faecal total VFA (g/kg), slurry total VFA (g/kg), average slurry pH and ammonia emission from slurry over 16-day measuring period (g) on the intake of non-starch polysaccharides (g/d)

Dependent variable	Constant	<b>8</b> 6	+1	β" ± SE′	R <sup>*</sup>	ě.,	
Faecal total VFA	3.339	0.004280	+1	0.004280 ± 0.000526	66.4	***	
Slurry total VFA	3.116	0.005091	+1	0.000978	54.1	***	
Average slurry pH	8.034	-0.001177	+I	0.000281	33.4	***	
Ammonia emission	3.166	-0.001608	+I	0.000163	74.6	**	
Regression coefficient.			Ł				

Kegu

bStandard error of regression coefficient.

<sup>c</sup>Correlation coeficience. <sup>d</sup>Probability: \*\*\**P* < 0.001.

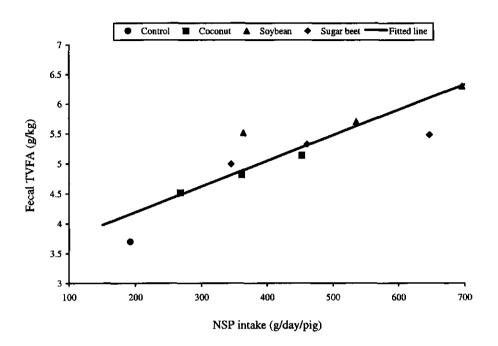


Figure 1. Total VFA concentrations of feces (g/kg) related to the daily intake of non-starch polysaccharides (g/day/pig)

The chemical composition of the slurry after the 16-day measuring period of ammonia emission for different diets is shown in Table 6. The source and level of dietary carbohydrates strongly influenced DM content of slurry (P < 0.001). The slurry from pigs fed the coconut expeller diet had the highest DM content (P < 0.001). Increasing the level of carbohydrates in the diet decreased the slurry DM (P < 0.001). The total N content of slurry was the highest on the coconut expeller diet. Ammonium contents were very similar for all the diets. The total VFA concentration in the slurry increased on average by 0.03, 1.10 and 0.60% for each per cent of cornstarch replaced with coconut expeller, soybean hulls and dried sugar beet pulp, respectively.

Table 7 shows the effect of the intake of dietary NSP on the total VFA concentration of faeces and slurry and on the average pH and the ammonia emission from slurry across all treatments. The total VFA concentrations of faeces (P < 0.001) and slurry (P < 0.001) were positively related to the intake of dietary NSP. The regression coefficient of the intake of NSP on the total VFA concentration of faeces is similar to that calculated for slurry.

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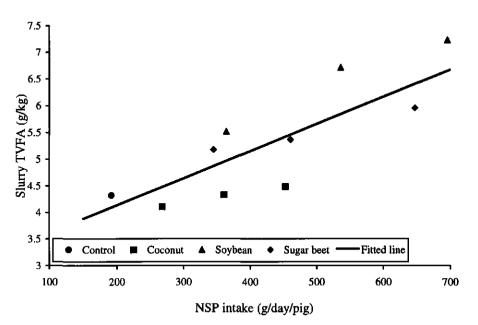
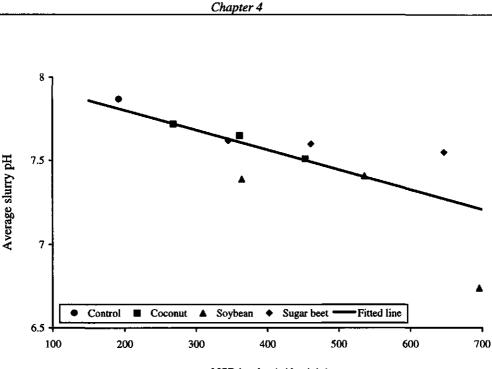


Figure 2. Total VFA concentrations of slurry (g/kg) related to daily intake of non-starch polysaccharides (g/day/pig)

The pH of slurry (P < 0.001) and the ammonia emission (P < 0.001) were negatively related to the intake of dietary NSP. In general, a high intake of dietary NSP increased the total VFA concentration (Figures 1 and 2), reduced the pH (Figure 3) and the ammonia emission (Figure 4) from slurry of pigs. Figures 3 and 4 show the effect of intake of dietary NSP on the ammonia emission from slurry is very similar to its effect on the average pH of the slurry. In Figure 5, the overall average time patterns of the pH at the different levels in the slurry and the ammonia emission from the slurry over the 16-day measuring period are illustrated. The ammonia emission from slurry decreased rapidly after the initial measurements until day 5 and then decreased at a slower rate. The pH at the different levels in the slurry showed a slight increase at the start of measuring and a steady decrease after day 4.



NSP intake (g/day/pig)

Figure 3. Average pH of the slurry related to the daily intake of non-starch polysaccharides (g/day/pig)

## Discussion

This investigation supports the hypothesis that fermentable carbohydrates in the diet can influence the ammonia emission from pig slurry. According to Sommer and Husted (1995), the slurry pH is of great importance for the ammonia emission from pig slurry. Because the effect of pH on ammonia emission is very strong, a minor change in pH can have a large effect. In this study, the source and the level of fermentable carbohydrates in the diet are important factors affecting the pH and the ammonia emission. Increasing the amounts of fermentable carbohydrates in the diet enhanced the microbial activities in the hind gut of pigs and in the slurry during storage increasing VFA formation in faeces and in the slurry. This lowered the pH of the slurry, thereby as a consequence, reducing the ammonia emission. This was confirmed by regression analysis of those factors on the intake of dietary NSP (Table 7). For each 100 g increase in the intake of dietary NSP, total VFA of faeces and slurry increased by about 0.43 and 0.51 g/kg, respectively. The average pH of the slurry decreased by about 0.12 unit and the ammonia emission from slurry decreased by 5.4%.

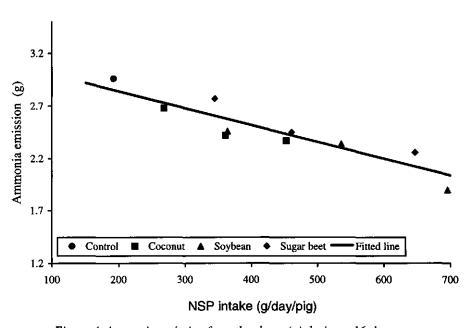


Figure 4. Ammonia emission from the slurry (g) during a 16-d storage period related to the daily intake of non-starch polysaccharides (g/day/pig)

If the daily intake of NSP reaches 700 g as the highest level of the soybean hulls or sugar beet pulp diets fed to the pigs, the ammonia emission from slurry will decrease by about 38%. The soybean hulls diet had the greatest effect on the reduction of pH and the ammonia emission. This can be explained by the highest intake level of NSP in pigs fed the soybean hulls diet. The effect of coconut expeller or dried sugar beet pulp on pH and ammonia emission were smaller compared to the effect of soybean hulls (Table 5). Faecal and slurry VFA concentrations were higher from pigs fed the dried sugar beet pulp diet compared with those fed diets with coconut expeller. This demonstrates that the pH change observed in this experiment was influenced by factors in addition to the VFA concentration in the slurry. According to Wellinger (1985) and Sommer and Husted (1995), both NH<sub>4</sub>-N and carbonate concentrations were very similar in slurry from pigs fed the coconut expeller and the dried sugar beet pulp diets.

Faecal output also differed among the different sources of carbohydrates. From Table 4, it is obvious that the faeces, which contained a higher concentration of VFA, were also produced in a larger amount. In this research, a fixed amount of faeces was mixed with a fixed amount of standard urine.

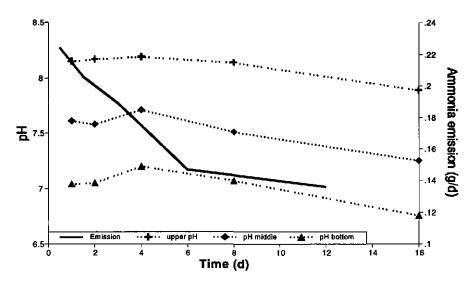


Figure 5. The pH patterns at different levels in the slurry and the ammonia emission from slurry during the 16-d storage period in the in vitro experiment

Canh et al. (1997a) reported that, at the same water intake, increasing the level of fermentable carbohydrates in the diet of pigs increases the amount of faeces and decreases the amount of urine. Therefore, in a practical situation, the concentrations of VFA in the slurry will likely differ more among the different diets than illustrated in this research. The pigs fed a high-NSP diet produce more faeces with a higher VFA concentration than the pigs fed a low-NSP diet. These faeces will be mixed with a lower amount of urine. Therefore, if more fermentable carbohydrates are included in the diet, a further reduction of ammonia emission from the slurry can be expected in real pig houses, because of an additional effect of a shift in N excretion from urine to N in faeces. When more fermentable carbohydrates are included in the form of bacterial protein and less via the urine in the form of urea (Morgan and Whittemore, 1988; Mroz et al., 1993; Schulze et al., 1995; Canh et al., 1997a). The conversion of urea into ammonia is a very rapid process in comparison with the breakdown of bacterial protein. This shift in nitrogen excretion will also lower the ammonia emission. Thus, it may be expected that in practice a larger effect can be expected than was observed in this study.

On average, the ammonia emission decreased by about 40% during the storage period of 16 day (extrapolated from the emission line, Figure 5). The calculated average  $NH_4$ -N concentration in the slurry decreased by 13%, from 2.55 to 2.33 g/kg over the 16-day measuring period. Therefore, the ammonia concentration in the slurry explains only a small part of the reduction in the gaseous ammonia emission during the storage period. There was a rapid release of ammonia from the upper layer of the slurry into

the air initially. At the same time ammonia from the lower layers of slurry diffused to the upper layer. Thus, in addition to the reduction of ammonia concentration, the slow diffusion of ammonia from the lower layers to the surface of slurry might also have contributed to the reduction of ammonia emission.

In this experiment, the sources of the fermentable carbohydrates were chosen on the basis of differences in the composition of the cell walls. On the basis of the cell wall fractions analysed, the different components of the cell walls in faeces can be estimated (Van Soest, 1967). The faeces from pigs fed the soybean hulls diets contained more fermentable fractions such as cellulose than those obtained from pigs fed the other diets. Also the high lignin content excreted in faeces of pigs fed the coconut expeller diet may have a negative effect on the fermentative process in the slurry during storage. This resulted in a slow production of slurry VFA. High concentrations of the easily fermentable carbohydrates, cellulose and hemicellulose, in combination with a low concentration of lignin, seem to promote the highest rate of fermentation and the highest concentrations of VFA. Our results agree with those of Mroz et al. (1993) and Canh et al. (1997a, 1997b), who observed a decrease in slurry pH and ammonia emission with increasing dietary levels of cellulose and hemicellulose and decreasing dietary levels of cellulose and hemicellulose and decreasing dietary level of lignin.

In this research, faeces were mixed with a standardised urine and the ammonia emission was measured with an in vitro laboratory system. However, the in vitro system is in relative static conditions as compared with the dynamic situation with a constant addition of fresh urine and faeces in practical pig buildings. The inclusion of fermentable carbohydrates in the pig's diet may be a practical method for ammonia emission control. However, this effect on the ammonia emission needs to be verified in practical situations. Nevertheless, in this research the potential impact of the faeces derived from these different diets on the microbial metabolism to form VFA in slurry has been demonstrated.

## Conclusion

The level and the source of fermentable carbohydrates in pig diets significantly influence the volatile fatty acid concentration of faeces and slurry, and the pH and the ammonia emission from the slurry. The inclusion of soybean hulls in the diet produces higher concentrations of volatile fatty acids in faeces and slurry, a lower pH of the slurry and a lower emission of ammonia from the slurry compared with coconut expeller and dried sugar beet pulp. The addition of soybean hulls may be a practical and economical method to reduce the ammonia emission from pig houses to environmentally acceptable levels.

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## **Chapter 5**

# Effect of Non-starch Polysaccharide-rich By-product Diets on Nitrogen Excretion and Nitrogen Losses from Slurry of Growing-Finishing Pigs

T. T. Canh<sup>a,b,c,d,\*</sup>, M.W.A. Verstegen<sup>b</sup>, N. B. Mui<sup>a,b</sup>, A.J.A. Aarnink<sup>c</sup>, J. W. Schrama<sup>b</sup>, C. E. van't Klooster<sup>c</sup> and N. K. Duong<sup>a</sup>

<sup>a</sup>Department of Animal Science, University of Agriculture and Forestry, Hue, Vietnam. <sup>b</sup>Wageningen Institute of Animal Science (WIAS), Departments of Animal Nutrition and Animal Husbandry, Wageningen Agricultural University, The Netherlands.

<sup>6</sup>Department of Livestock Engineering, Institute of Agricultural and Environmental Engineering (IMAG-DLO), Wageningen, The Netherlands,

<sup>d</sup>Netherlands Foundation for the Advancement of Tropical Research

<sup>\*</sup>Tel. (31) 317 476552; Fax: (31) 317 425670; E-mail: T.T.Canh@IMAG.DLO.NL

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## Abstract

An experiment was conducted to investigate the effect of diet for growing-finishing pigs with high level of non-starch polysaccharides (NSP) from by-products on nitrogen excretion and nitrogen losses from slurry during storage. Sixteen commercial crossbred barrows of about 68 kg BW were randomly allotted to one of four diets. The control diet was formulated using tapioca and rice as basal energy sources. In the other diets, tapioca was replaced by either coconut expeller, rice bran or beer by-product. The diets differed mainly in the amount and composition of NSP. After a 12-day adaptation period, urine and faeces were collected separately in metabolism cages for 9 days. Urine and faeces from the first four days were used to analyse the nitrogen partitioning. Urine and faeces from the last 5 days were mixed as slurry. The slurry was sampled at the end of the collection period and again after 30 days storage, to analyse for nitrogen to calculate the losses.

Increasing dietary NSP reduced urinary nitrogen and nitrogen losses from the slurry during storage. The pigs fed the diet based on beer by-product excreted the most nitrogen via faeces and the least nitrogen via urine. Nitrogen losses from slurry of pigs fed the beer by-product were from 34 to 65% lower than from the other three diets.

It is concluded that including NSP-rich by-products in the diet of growing-finishing pigs reduces urinary nitrogen excretion and nitrogen losses from slurry during storage.

## Key Words: Pig, By-product, Polysaccharides, Nitrogen, Slurry, pH

## Introduction

Farms in Vietnam are generally mixed, with both crop and animal husbandry (Hai and Nguyen, 1997). Pig production is not only a major protein source for human consumption but the manure from it is also important in supplying organic fertiliser. It has long been known that much nitrogen is lost while manure is being stored or spread on arable land (Muck and Steenhuis, 1981, Sommer and Thomsen, 1993). Volatilisation of ammonia is the main cause of these nitrogen losses (Muck and Steenhuis, 1981, Maeda and Matsuda, 1997). Ammonia lost from slurry not only reduces fertiliser value,

but may also cause pollution of ground water and air pollution (Freney *et al.*, 1983, Apsimon and Kruse-Plass. 1990). The ammonium content and pH of slurry are important factors influencing the ammonia volatilisation from the slurry (Freney *et al.*, 1983, Canh *et al.*, 1997). According to Jongbloed and Lenis (1992), of the total ingested nitrogen by pigs, about 20% is excreted via faeces and about 50% in urine. Nitrogen excreted via faeces is predominately incorporated in bacterial protein, which is less susceptible to rapid decomposition. Nitrogen excreted via urine is mainly in the form of urea, which is easily converted into ammonia and carbon dioxide by the enzyme urease present in faeces. There are two basic ways to reduce the nitrogen excretion in pig urine: 1) by reducing nitrogen content of the diet (Spieker, 1992; Gatel and Grosjean, 1992, Kay and Lee, 1997). 2) by shifting nitrogen excretion from urine to faeces by including non-starch polysaccharides (NSP) in the diet (Schulze *et al.*, 1993, Canh *et al.*, 1997). Furthermore, NSP may influence the pH and ammonia volatilisation from slurry by volatile fatty acid (VFA) formation (Canh *et al.*, 1996 and 1997). So far, however, the concept of including NSP-rich by-products in the diet to reduce nitrogen losses from the slurry has not been fully explored. Therefore, the objective of this study was to investigate the effect of NSP-rich diets for growing-finishing pigs based on by-products on nitrogen excretion and nitrogen losses from slurry during storage.

## Material and methods

#### Animals and housing

A total of 16 commercial crossbred barrows (Vietnamese Mongcai × Large white), with initial BW of  $67.92 \pm 0.58$  kg were randomly allotted to one of four diets (Table 1). From 60 kg onwards the animals were kept in groups and were fed treatment diets. When the animals reached 65 kg body weight, they were housed individually in a controlled room in metabolism cages that allowed the separate collection of urine and faeces. Sizes of the metabolism cages were  $1.2 \times 0.6$  m (length × width) and were made of steel with wooden slats. The 21-day experimental period consisted of a 12-day adaptation period to allow the pigs to become accustomed to the cages and to the new diet and a 9-day period during which urine and faeces were collected. The average ambient temperature was about 27 <sup>0</sup> C and the average humidity was about 72%.

## Diets and feeding

The ingredient compositions of the experimental diets are given in Table 1. The control diet was composed with rice and tapioca as basal energy sources. In the other three diets, tapioca was partly exchanged with the same amount (200 g/kg diet) of coconut expeller, rice bran or beer by-product. Thus, the diets were similar, except for the contents of NSP-rich by-products and tapioca. The diet based on beer by-product had the highest NSP content (20.04%), followed by the diets based on rice bran (13.97%), coconut expeller (13.65%) and the tapioca (6.82%), respectively (Table 2).

Ingredients		Die	ets	
(as fed basis)	Tapioca	Coconut expeller	Rice bran	Beer by-product
Rice	231.5	231.5	231.5	231.5
Tapioca	509.5	370.0	340.0	353.0
Cane molasses	25.0	25.0	25.0	25.0
Groundnut extracted	125.0	100.0	125.0	100.0
Fish meal	78.0	35.0	48.0	25.0
Coconut expeller		200.0		
Rice bran			200.0	
Beer by-product				200.0
Chalk	11.5	11.5	11.5	11.5
CaHPO₄	4.0	4.0	4.0	4.0
KHCO <sub>3</sub>	2.5		2.0	7.0
Salt	3.0	3.0	3.0	3.0
Premix <sup>a</sup>	10.0	10.0	10.0	10.0

#### Table 1. Ingredient composition of the experimental diets (g/kg diet)

<sup>a</sup>The vitamin and mineral premix supplied per 1 kg feed: 9000 IU vitamin A, 1800 IU vitamin D<sub>3</sub>, 40 mg vitamin E, 3 mg vitamin K, 5 mg riboflavin, 50 mg ascorbic acid, 12 mg d-pantothenic acid, 30 mg niacin amide, 350 mg choline-chloride, 40 : g vitamin B<sub>12</sub>, 1 mg folic acid, 0.1 mg biotin, 0.5 mg C<sub>0</sub>; 0.06 mg Se, 0.4 mg J, 80 mg Fe, 25 mg Cu, 44 mg MnO<sub>2</sub>, 73 mg Zn, 20 mg Tylosin.

The diet based on beer by-product contained the highest fermentable fractions (cellulose and hemicellulose) of NSP and the diet based on coconut expeller contained the highest lignin content.

The pigs were fed 2.5 times the energy required for maintenance, which was assumed to be 294 kJ net energy per kg of metabolic weight  $BW^{0.75}$  (ARC, 1981). The ration was increased each day based on an estimated weight gain of 600 g/day. Feed was mixed with water before feeding (2 l/kg feed) and provided in two equal meals per day. Water was given ad libitum through a drinking nipple at the front of each metabolism cage.

## Measurements

To avoid disturbing the animals during the 9-day collection period, pigs were weighed four days before and one day after this period, before the morning feeding. The urine and faeces from each pig were collected separately and weighed twice daily. Urine was collected in a closed bucket (covered by lid with a central hole) via a funnel under the cage. Faeces were collected in a plastic bag ( $15 \text{ cm} \times 30 \text{ cm}$ ) using the velcro<sup>®</sup> support system (Van Kleef *et al.*, 1994). A piece of glasswool was placed in the funnel and a piece of fine-meshed gauze was placed over the urine bucket, to trap particulate. The urine buckets and faeces bags were replaced twice a day. The urine funnels was changed every morning and the amount of urine remaining in the glasswool was determined by weighing the glasswool.

Composition		Die	ts	····· - ···
(as fed basis)	Таріоса	Coconut expeller	Rice bran	Beer by-product
NE, kcal/kg	2312	2213	2318	2104
CP <sup>a</sup> , %	12.78	13.89	12.59	12.17
Crude fat <sup>a</sup> , %	2.49	4.39	4.47	3.07
NSP <sup>a,b</sup> , %	6.82	13.65	13.97	20.04
Cellulose <sup>a</sup> , %	2.53	2.20	3.33	5.26
Hemicellulose, %	0.86	0.66	1.94	6.13
NDF <sup>ª</sup> , %	4.17	3.36	5.98	12.32
ADF <sup>a</sup> , %	3.31	2.70	4.04	6.19
ADL <sup>a</sup> , %	0.67	1.69	1.08	1.31
Water <sup>a</sup> , %	13.89	13.12	13.68	12.30
Crude ash <sup>*</sup> , %	5.06	7.31	5.59	4.92
Starch and sugar <sup>a</sup> , %	58.96	47.64	49.70	47.50
Lysine, %	0.70	0.53	0.67	0.68
Methionine, %	0.27	0.24	0.25	0.24
Phosphorus, %	0.44	0.43	0.66	0.37
Sodium, %	0.21	0.17	0.18	0.16
Calcium, %	0.93	0.78	1.25	0.78
Potassium, %	0.83	0.97	0.87	0.82
Chloride, %	0.40	0.44	0.35	0.30
Magnesium, %	0.13	0.17	0.27	0.12
Copper, ppm	3.70	3.60	3.10	3.30
dEB <sup>c</sup> , meq/100g	19.20	19.93	19.93	19.65

Table 2. Chemical composition of experimental diets

<sup>a</sup> Analysed.

<sup>b</sup> Non-starch polysaccharides, Determined as organic mater – (crude protein + crude lipid + starch + sugar). <sup>c</sup>Dietary electrolyte balance (calculated as meq Na + K - Cl).

The urine and faeces collected in the first 4 days were used for determining the nitrogen balance. They were stored at -20 °C until the nitrogen analyses were performed. To prevent nitrogen being lost by ammonia volatilisation during this period, urine was collected in 50 ml of 25% sulphuric acid to keep the pH below pH 2. The urine and faeces collected during the last 5 days, without adding acid to the urine, were used to make slurry. Urine and faeces were collected twice a day, their pH was measured directly after the collection, and they were then mixed to slurry in a plastic bucket with a surface area of 0.3 m<sup>2</sup>. The buckets were stored uncovered in a room at ambient temperature (average about 27 °C). After the collection period the pooled slurry was sampled for chemical analyses. The buckets were kept for a further 30 days in the same room. After this storage period, the slurry was mixed and sampled for chemical analysis. The slurry was weighed at the beginning and the end of the storage period. The differences in total nitrogen of slurry at the start and the end of this 30-day storage period were used to calculate the nitrogen losses from the slurry.

### Chemical analysis

All samples were analysed in duplicate. The diets and excreta were analysed for DM, ash, crude fibre, crude fat, and total N according to AOAC (1990).  $NH_4^+$ -N content was determined titrimetrically and urinary urea was determined by kinetic UV test according to Neumann and Ziegenhorn (1977). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed as described by Huisman (1990). The pH was measured at room temperature with a Sentron instrument glass electrode (model 1001) directly submerged in the urine, in diluted faeces (mixed with distilled water in a ratio 1:4) and in the slurry.

#### Statistical analysis

The individual pig was the experimental unit. Effect of the diet on average daily gain, nitrogen intake, nitrogen retention, nitrogen excretion, and apparent faecal nitrogen digestibility, and on excreta compositions and nitrogen losses were analysed by one-way ANOVA using the GENSTAT statistical package (Genstat 5 Committee, 1993). When an F-test showed a significant effect of diet (P < 0.05), means were separated with the LSD procedure with a confidence level of 0.05 (Genstat 5 Committee, 1993).

## Results

#### Nitrogen intake and nitrogen excretion

No health problems occurred during the experimental period, and no feed refusals were observed. Table 3 shows the animal daily BW gain, nitrogen intake, nitrogen excretion, nitrogen retention and apparent faecal nitrogen digestibility of the pigs on the different diets. Diet did not affect daily gain (P > 0.05). Daily nitrogen intake from pigs fed the coconut expeller and beer by-product based diets was higher than from pigs on the other two diets (P < 0.05). Total nitrogen excretion was higher in the coconut expeller based diet than in the beer by-product based diet (P < 0.05). The nitrogen excretion pattern, which is indicated by the ratio of urinary nitrogen to faeces nitrogen, differed considerably between diets (P < 0.001). The pigs fed the beer by-product diet excreted more nitrogen in faeces and less nitrogen in urine (P < 0.001). Faecal nitrogen was lowest in the tapioca-based diet. The urinary nitrogen excretion was highest in pigs fed the tapioca and coconut expeller based diets and lowest in pigs fed the tapioca has diets and lowest in pigs fed the tapioca has been by-product based diets and lowest in pigs fed the tapioca has been by-product based diets and lowest in pigs fed the tapioca has been by-product based diets and lowest in pigs fed the tapioca has been by-product based diets and lowest in pigs fed the tapioca has been by-product based diets and lowest in pigs fed the tapioca has been by-product based diets and lowest in pigs fed the tapioca has been by-product based diets and lowest in pigs fed the tapioca has by by-product based diets and lowest in pigs fed the tapioca has by by-product based diets and lowest in pigs fed the diet based on been by-product (P < 0.001). The pigs fed the been by-product based diets and lowest in pigs fed the diet based on been by-product (P < 0.001). The pigs fed the been by-product based diets and lowest in pigs fed the diet based on been by-product (P < 0.001). The pigs fed the been by-product based diet retained more nitrogen than the pigs

Variable						
	Таріоса	Coconut Expeller	Rice bran	Beer by- product	<i>P</i> <sup>4</sup>	SEM
Number of animals	4	4	4	4		
Initial BW, kg <sup>b</sup>	68.4	67.6	67.6	67.7	NS	1.51
Final BW, kg <sup>c</sup>	76.9	77.1	76.9	77.0	NS	2.43
Weight gain, g/dayd	607	679	631	661	NS	41
N intake, g/day	37.1°	40.9 <sup>f</sup>	35.5°	41.6 <sup>f</sup>	*	1.10
Faecal N, g/day	4.42 <sup>e</sup>	5.06 <sup>f</sup>	5.52 <sup>f</sup>	8.03 <sup>8</sup>	***	0.17
Urinary N, g/day	18.4 <sup>e</sup>	20.2 <sup>f</sup>	15.8 <sup>8</sup>	11.7 <sup>h</sup>	***	0.39
Tot.N excretion, g/day	22.8 <sup>eg</sup>	25.3°	21.3 <sup>eg</sup>	19.7 <sup>fg</sup>	*	1.45
Urinary N:faecal N	4.16 <sup>e</sup>	4.00 <sup>e</sup>	2.86 <sup>f</sup>	1.46 <sup>g</sup>	***	0.14
Apparent N digestibility, %	88.1 <sup>e</sup>	87.6 <sup>e</sup>	84.5 <sup>f</sup>	80.7 <sup>g</sup>	***	0.81
N retention, % of intake	38.5°	38.1°	40.0 <sup>e</sup>	52.6 <sup>r</sup>	**	2.40

# Table 3. Weight gain, nitrogen intake, excretion, apparent nitrogen digestibility and retention of pigs fed different diets

<sup>a</sup>Probability of a significant treatment effect. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; NS = not significant. <sup>b</sup>The pigs were weighed four d before the collection period.

"The pigs were weighed one d after the collection period.

<sup>d</sup>Calculated for a period of 14 d between the initial BW and the final BW.

effigib Different letters in superscript indicate significant difference (P < 0.05).

#### Amount, pH and composition of urine and faeces

The chemical composition of urine and faeces from pigs fed the different diets is shown in Table 4. The amount of faeces and urine differed significantly between diets (P < 0.01). The pigs fed the beer by-product based diet produced more faeces and less urine than the pigs on the other diets. No differences in faecal and urinary amounts were found between the other three diets. The diet had a profound effect on urinary urea concentration (P < 0.001). The total amount and the concentration of urinary urea was highest in the pigs fed the beer by-product based diet excreted 23 to 42% less urea in urine than the pigs on the other three diets. On average, the pH of faeces from pigs fed the beer by-product based diet was about 0.69 units lower than the pH of faeces from pigs fed the other three diets (P < 0.01). The pH of urine from pigs fed the coconut expeller based diet was highest. No significant difference in urinary pH was found between the other three diets.

#### Nitrogen losses from slurry during storage

Diet did not affect amount of slurry (P > 0.05). However, slurry DM was significantly influenced by the diet (P < 0.001). The DM concentrations of slurry increased considerably during storage, because water evaporation. They were highest for the beer by-product based diet and lowest for the tapioca based

Component						
	Tapioca	Coconut expeller	Rice bran	Beer by- Product	$P^{a}$	SEM
DM, g/kg						
Faeces	392 <sup>b</sup>	458°	408 <sup>b</sup>	350 <sup>d</sup>	**	11.1
NH4-N, g/kg						
Faeces	0.53 <sup>b</sup>	0.62°	0.57 <sup>b</sup>	0.54 <sup>6</sup>	**	0.02
Urine	0.32 <sup>b</sup>	0.23°	0.31 <sup>b</sup>	0.34 <sup>b</sup>	***	0.01
Total N, g/kg						
Faeces	8.87 <sup>b</sup>	10.43°	10.43 <sup>c</sup>	9.54 <sup>60</sup>	***	0.47
Urine	4.83 <sup>b</sup>	5.28°	4.18 <sup>d</sup>	3.75°	***	0.11
Faecal ash, g/kg	104.5 <sup>6</sup>	134.1°	106.3 <sup>b</sup>	68.4 <sup>d</sup>	***	6.67
Faecal CF, g/kg	67.6 <sup>be</sup>	76.6 <sup>bc</sup>	77.5°	62.6 <sup>de</sup>	**	2.95
Urin. urea, mmol/l	142.4 <sup>6d</sup>	162.3 <sup>b</sup>	122.8 <sup>cd</sup>	115.7°	***	8.61
Tot. urinary urea, mmol/day	542 <sup>b</sup>	619°	464 <sup>d</sup>	357 <sup>e</sup>	***	13.3
pН						
Faeces	8.00 <sup>6</sup>	7.74 <sup>b</sup>	7.96 <sup>⁵</sup>	7.21	**	0.15
Urine	7.52 <sup>b</sup>	7.90 <sup>°</sup>	7.63 <sup>b</sup>	7.48 <sup>b</sup>	*	0.08
Amount, g/d						
Faeces	498 <sup>b</sup>	485 <sup>b</sup>	529 <sup>b</sup>	842°	**	21.5
Urine	3806 <sup>b</sup>	3815 <sup>b</sup>	3780 <sup>6</sup>	3086°	**	175

Table 4. Amount and composition of faeces and urine from pigs fed different diets

<sup>a</sup>Probability of a significant treatment effect: \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; NS = not significant. <sup>b,c,d,e</sup>Different letters in superscript indicate significant difference (p < .05).

diet (Table 5). Diets did not influence the total nitrogen concentration of the slurry at day 1 (P > 0.05). However, on day 30 total nitrogen concentration was lower on the tapioca and coconut expeller based diets than the other two diets (P < 0.01). On day 1, the pH of slurry from pigs fed the tapioca, the coconut expeller and the rice bran based diets was not different. The pH of slurry from pigs fed the beer by-product based diet was approximately 0.7 to 0.9 unit lower than the other three diets (P < 0.001). The pH of the slurry fell slightly during the storage period but differences between diets followed the same pattern of those observed on day 1. Total nitrogen losses from slurry during the 30-day storage period was remarkably different between diets. Nitrogen losses for the beer by-product, rice bran, coconut expeller and tapioca based diets were 5.39, 8.13, 13.84 and 15.30%, respectively. Nitrogen losses from slurry of pigs fed the beer by-product based diet were 34 to 65% lower than for the other three diets.

	Diet					
Component	Tapioca	Coconut expeller	Rice bran	Beer by-product	- P <sup>a</sup>	SEM
Day 1				······································		
Amount <sup>b</sup> , kg	20.52	20.50	19.49	18.54	NS	2.01
DM, g/kg	74.53 <sup>4</sup>	78.05 <sup>₫</sup>	81.51 <sup>d</sup>	99.37°	***	2.27
Total N, g/kg	5.24	5.54	5.25	5.01	NS	0.28
pH	8.07 <sup>d</sup>	7.88 <sup>d</sup>	8.01 <sup>d</sup>	7.19 <sup>e</sup>	***	0.12
Day 30						
Amount, kg	14.37	14.09	13.12	12.87	NS	1.27
DM, g/kg	104.43 <sup>d</sup>	109.78 <sup>de</sup>	118.06 <sup>e</sup>	140.90 <sup>r</sup>	***	3.12
Total N, g/kg	6.34 <sup>d</sup>	6.04 <sup>d</sup>	7.16 <sup>e</sup>	6.87 <sup>e</sup>	**	0.17
рН	7.89 <sup>d</sup>	7.77 <sup>d</sup>	7.83 <sup>d</sup>	7.17°	***	0.09
N losses (%) <sup>c</sup>	15.30 <sup>d</sup>	13.84 <sup>d</sup>	8.13 <sup>e</sup>	5.39 <sup>f</sup>	***	1.11

<sup>a</sup>Probability of a significant treatment effect: \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; NS = not significant. <sup>b</sup>Amount of 5 collection days.

calculated by comparing the total N of slurry between day 30 and day 1.

<sup>d.e.f</sup>Different letters in superscript indicate significant difference (P < 0.05).

#### Discussion

The main objective of this study was to evaluate the possibility to lower the pH of slurry and to lower urinary nitrogen excretion, and nitrogen losses from slurry during storage by increasing the level of dietary NSP from NSP-rich by-products.

#### Nitrogen excretion

In the present study the diets had a similar NE content but were formulated from different byproducts and differed mainly in NSP content. The results from this experiment support the concept that the amount of NSP in the diet can influence the nitrogen excretion pattern in pigs. Increasing the amount of NSP in the diet shifts nitrogen excretion via urine to faeces. The largest effect was obtained from the diet based on beer by-product. This diet contained the highest amount of NSP and fermentable fractions of NSP in the diet, compared with the other three diets. The difference in nitrogen excretion pattern in this study is in agreement with our previous observations (Canh *et al.*, 1996 and 1997) and with the findings of other researchers (Mroz *et al.*, 1993, Schulze *et al.*, 1993) who found that the total amount of NSP as well as contents of cellulose and hemicellulose were related positively to faecal nitrogen excretion and negatively to the urinary nitrogen excretion. Fermentable carbohydrates serve as an energy source for microflora in the large intestine of pigs, and urea secreted from the blood into the large intestine (Low, 1985) serves as a nitrogen source. A high-energy supply of fermentable organic matter to the microbes of the large intestine induces a high secretion of urea from the blood (Low, 1985) and a high microbial growth. When urea is transferred to the lumen of the large intestine, it is broken down to ammonia by bacterial urease and then used for microbial protein synthesis. This protein is finally excreted in the faeces (Canh *et al.*, 1997). The amount of urea secreted from blood into the large intestine increases with increased dietary fibre content (Low, 1985), resulting in a reduced urea content in the portal plasma (Malmlöf, 1985). The synthesis of microbial protein causes less ammonia to be reabsorbed from the colon. As a result, nitrogen excretion shifts from urine to faeces.

When comparing the two diets with similar contents of NSP, urinary nitrogen excretion from pigs fed the coconut expeller based diet was higher than observed from the pigs fed the rice bran based diet. This difference might be caused by the higher intake of nitrogen by pigs fed the coconut expeller. The high lignin content of this diet might also depress microbial activities and reduce the degradation of fibre in the large intestine of pigs (Canh, *et al.*, 1997).

#### Nitrogen loss from slurry during storage

The results from this experiment support our recent studies (Canh *et al.*, 1996 and 1997) which have shown that fermentable carbohydrates in the diet can influence the ammonia volatilisation from pig slurry during storage. In the present study, the level of NSP in the diets ranged from 6.82 to 20.04%. It was observed that the total nitrogen losses from the slurry during the 30-day storage were lower for the NSP-rich diets. In this experiment, the slurry was stored in plastic containers. Nitrogen losses from the slurry only occurred by ammonia volatilisation from the surface of the slurry. According to Muck and Steenhuis (1981), the main part of ammonia emission originates from urea in the urine. Urea is converted into ammonia and carbon dioxide by urease present in faeces. The conversion starts as soon as the urine comes into contact with faeces. In the slurry, the equilibrium vapour pressure of ammonia is controlled by the total concentration of ammoniacal nitrogen and the pH of slurry.

In the present study, two main reasons for the lower volatilisation of ammonia from slurry of pigs fed the NSP-rich diets can be hypothesised. Firstly, increasing the amount of NSP in the diet caused nitrogen excretion to shift from urine to faeces. This resulted in a reduction of urinary urea content, and consequently, reduced the ammonium content of slurry (Schulze *et al.*, 1993; Canh *et al.*, 1997). Secondly, the pH of slurry was lowered when more NSP was included in the diet. According to Sommer and Husted (1995), the slurry pH is very important for the government of ammonia volatilisation from pig slurry. In our previous studies (Canh *et al.*, 1996 and 1997) we found that the pH of the slurry was strongly influenced by the ammonium and VFA concentrations of the slurry. In pigs, VFA are mainly produced from dietary NSP by anaerobic microbial fermentation in the large intestine (Imoto and Namioca, 1978, Canh *et al.*, 1997) and in the slurry during storage (Spoelstra, 1979, Canh *et al.*, 1996 and 1997). In this study, VFA formation and the reduction of slurry ammonia probably caused the low pH of slurry from pigs fed NSP-rich diets. A lower ammonium concentration and a lower pH of slurry reduced the losses of nitrogen through evaporation.

# Conclusions

Including non-starch polysaccharides rich by-products in the diet of pigs shifts nitrogen excretion from the volatilisable form in urine to the less accessible protein form in the faeces. Non-starch polysaccharides also lower the pH of slurry, consequently there are clearly reduced nitrogen losses from slurry during storage. Such an approach may be an economical way of improving the quality of fertiliser from pig farming and of reducing the environmental impact of pig production.

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# **Chapter 6**

# Effect of dietary fermentable non-starch polysaccharides From pressed sugar beet pulp silage on ammonia emission from slurry of growing-finishing pigs

T. T. Canh<sup>a,b,c,\*</sup>, J. W. Schrama<sup>b</sup>, A.J.A. Aarnink<sup>a</sup>, M.W.A. Verstegen<sup>b</sup>, C. E. van't Klooster<sup>a</sup> and M.J.W. Heetkamp<sup>b</sup>

<sup>a</sup>Department of Livestock Engineering, Institute of Agricultural and Environmental Engineering (IMAG-DLO), Wageningen, The Netherlands.

<sup>b</sup>Wageningen Institute of Animal Science (WIAS), Departments of Animal Nutrition and Animal Husbandry, Wageningen Agricultural University, The Netherlands.

<sup>°</sup>Netherlands Foundation for the Advancement of Tropical Research.

<sup>\*</sup>Tel. (31) 317 476552; Fax: (31) 317 425670; E-mail: T.T.Canh@IMAG.DLO.NL

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et al., 1997). Furthermore, fermentable non-starch polysaccharides (NSP) can decrease the pH of slurry by volatile fatty acid (VFA) formation (Paul and Beauchamp, 1989; Farnworth et al., 1995; Canh et al., 1996). Recently, Canh et al. (1996) found a reduction of ammonia emission from the slurry of pigs fed dried sugar beet pulp. Sugar beet pulp contains a considerable amount of NSP which can be used as an energy source for the pigs (Longland and Low, 1988; Chabeauti et al., 1991). The sugar beet pulp NSP is easily fermentable in the hind gut of pigs because of its low lignin content and a considerable level of pectin. Researchers interested in incorporating sugar beet pulp into pig diets have proposed possible benefits of the end products (VFA) of NSP fermentation. So far, however, the concept of manipulation of NSP with sugar beet pulp silage (SBPS) in the diet to reduce the pH and ammonia emission from the slurry has not been fully explored. Therefore, the objective of this study was to investigate the effect of dietary NSP originating from SBPS on the pH and ammonia emission from slurry of growing-finishing pigs.

## Materials and methods

#### Housing, animals and experimental design

Commercial crossbred barrows [Great Yorkshire × (Dutch Landrace × Great Yorkshire)], with initial body weight of  $45.3 \pm 0.57$  kg, were used in a completely randomised design to evaluate the effect of dietary NSP from sugar beet pulp silage (SBPS) on the pH and ammonia emission from slurry of growing-finishing pigs. Twelve groups of 14 barrows were assigned to one of four diets conducted in six 20-day experimental series in two climate chambers. The diets differed in the amount of NSP by altering the level SBPS. The control diet contained no SBPS. In the other diets, tapioca was replaced with three levels of SBPS: 5, 10, and 15% of SBPS (on dry matter basis)(Table 1). Thus, the diets were identical, expect for the contents of SBPS and tapioca. In the diet starch was replaced by NSP by the exchange of tapioca by SBPS. Pigs were housed in two subgroups of 7 in two pens in each of two climate chambers (Verstegen *et al.*, 1987). Each pen had a  $8.3 \text{-m}^2$  floor area, including a slurry channel (at the front side of the pen) connected with a drainage pipe and valve for the removal of slurry. The size of the slurry channel running along the two pens, was  $5.75 \times 0.50 \times 0.38$  m (length × width × depth). The channel was covered with metal slats. The temperature in the chambers was set at 20 °C and the relative humidity at 65%.

The pigs were fed 2.5 times the energy required for maintenance (Table 2), which was assumed to be 440 kJ ME/kg of metabolic weight  $BW^{0.75}$  (ARC, 1981). The amount of feed was increased daily based on a predicted weight gain of 500 g/d. Feed was mixed with water (2.2 L/kg of feed DM) in a trough and provided in two equal meals per day at 8.00 and 16.00 h. Water was given ad libitum through two drinking nipples in each pen. The different diets were sampled while they were being weighed out during the 7-day collection period. The daily samples were pooled and the subsamples were taken for the analyses of nutrient composition.

82

Ingredients (%)	0%-SBPS <sup>a</sup>	5%-SBPS <sup>a</sup>	10%-SBPS*	15%-SBPS*
Constant and a strand	0.00	E	11.11	16 67
Sugar beet pulp silage <sup>b</sup>	0.00	5.56	11.11	16.67
Tapioca	35.85	30.29	24.74	19.18
Wheat	20.00	20.00	20.00	20.00
Wheat bran	15.00	15.00	15.00	15.00
Soya bean meal	23.00	23.00	23.00	23.00
Soya bean oil	2.00	2.00	2.00	2.00
CaCO <sub>3</sub>	1.10	1.10	1.10	1.10
CaHPO₄	0.70	0.70	0.70	0.70
NaCl	0.30	0.30	0.30	0.30
L-Lysine	0.03	0.03	0.03	0.03
DL-Methionine	0.02	0.02	0.02	0.02
Premix <sup>c</sup>	1.00	1.00	1.00	1.00
Diamol	1.00	1.00	1.00	1.00

Table 1. Ingredient composition of the experimental diets

<sup>a</sup>Calculated on DM basis

<sup>b</sup>In the calculation of the diet, the DM content of SBPS was fixed at 90%.

<sup>o</sup>The vitamin and mineral premix supplied per 1 kg feed: 9000 IU vitamin A, 1800 IU vitamin D<sub>3</sub>, 40 mg vitamin E, 3 mg vitamin K, 5 mg riboflavin, 50 mg ascorbic acid, 12 mg d-pantothenic acid, 30 mg niacin amide, 350 mg choline-chloride, 40 :g vitamin B<sub>12</sub>, 1 mg folic acid, 0.1 mg biotin, 0.5 mg Co; 0.06 mg Se, 0.4 mg J, 80 mg Fe, 25 mg Cu, 44 mg MnO<sub>2</sub>, 73 mg Zn, 20 mg tylosin.

The 20-day experimental period consisted of a 13-day adaptation period to allow the pigs to become accustomed to the pen, the chamber, and their new diet, and a 7-day period during which slurry was collected. Before and after the collection period, the chambers were cleaned with water. After the 7-day collection period the pH of slurry in the pit was measured at the top layer and 10 cm below the slurry surface. The slurry was then mixed, the pH of mixed slurry was measured and samples were taken for chemical analyses and for the in vitro measurement of ammonia emission. After each experimental series the chambers were additionally disinfected with formalin.

#### Measurements

The pigs were weighed at days 1 and 20 of the 20-day experimental period, before the morning feeding. Grab samples of fresh faeces were collected for 5 days in the morning, during the 7-day slurry collection periods. The samples of faeces were pooled and stored at -20 °C. At the end of the collection period, faeces were mixed and sub-samples were taken to analyse the nitrogen and acid-insoluble ash contents. The apparent faecal nitrogen digestibility was determined by marker method, using acid-insoluble ash as a marker.

The ammonia emission was determined in vitro in a laboratory set up at 20 °C for 7 days according to the procedures described by Derikx and Aarnink (1993). Slurry (2 kg) was placed in a 6.5 l vessel covered by a lid. The surface area of the slurry in the vessel was  $284 \text{ cm}^2$ .

#### Chapter 6

Composition (% of DM)	0%-SBPS <sup>a</sup>	5%-SBPS"	10%-SBPS*	15%-SBPS <sup>a</sup>
Crude protein	18.53	18.97	19.61	19.99
Crude fat	3.71	3.72	3.78	3.80
Crude fibre	5.19	6.07	6.96	7.83
Starch	41.47	38.19	34.14	31.38
NSP <sup>b</sup>	27.50	30.46	33.98	36.54
Sugar <sup>c</sup>	0.69	0.64	0.53	0.36
Ash	8.01	8.02	7.96	7.91
NE <sup>d</sup> , Mj/kg	10.47	10.33	10.19	10.06
Lysine	0.98	1.01	1.05	1.08
Methione + Cystine <sup>d</sup>	0.60	0.61	0.63	0.65
Ca <sup>d</sup>	0.80	0.82	0.84	0.86
P <sup>d</sup>	0.66	0.66	0.66	0.66
DEB <sup>e</sup> ,meq/100g	25.11	24.72	24.33	23.93

Table 2. Nutrient compositions of the experimental die
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\*Calculated on DM basis

<sup>b</sup>Non-starch polysaccharides, determined as organic matter - (crude protein + crude lipid + starch + sugar) <sup>°</sup>Amount of free mono-saccharides and di-saccharides

<sup>d</sup>Calculated from CVB (1994)

<sup>e</sup>Dietary electrolyte balance (dEB; calculated as meq Na + K - Cl)

Two vessels were used for each treatment in each experimental series. Air entered the vessel through small holes at the edge of the lid and left the vessel in the centre. Ammonia in the outgoing air was removed by passing the airflow through two impingers, each containing 70 ml HNO<sub>3</sub> (0.5 M). The second impinger served as a control and contained no more than 5% of the ammonia trapped in the first impinger. The air left the system after passing a water trap, an air flow controller adjusted to 4.2 l/min and a pump. The first impinger was replaced at days 1, 2, 4 and 7. The second impinger was replaced at days 4 and 7. From the first and the second impingers both the ammonia concentration and the volume of the liquid were determined. The ammonia emission was calculated by multiplying the volume by the ammonia concentration. The mean emission of the two slurry vessels was used in the statistical analysis. The pH of slurry was measured at the top, the middle and the bottom layers (1, 7 and 12 cm beneath the surface of the slurry column) during replacement of the first impingers.

#### Chemical analysis

All analyses on feed and faeces and slurry were performed in triplicate following AOAC (1990) procedures. The concentration of  $NH_4^+$ -N in slurry was determined titrimetrically according to NEN 3235 (NEN, 1983). Volatile fatty acids in slurry were measured with a Packard 427 gas chromatograph equipped with a flame ionisation detector (Derikx *et al.*, 1994). Data on NE, amino acids, dietary electrolyte balance (dEB) and minerals were obtained from CVB (1994), in which data on nutrient composition, digestibility and feeding value of feedstuffs have been presented along with the references

for determination of the chemical composition. The pH of slurry was measured at room temperature with a Sentron instrument glass electrode (model 1001) directly submerged in the slurry.

#### Statistical Analysis

The group was the experimental unit for all data. The effects of diet on N intake, N retention, apparent faecal N digestibility and slurry composition and on the pH and ammonia emission from slurry were analysed by one-way ANOVA using the GENSTAT software (Genstat 5 committee, 1993). When an F-test showed a significant effect of the diet (P < 0.05), means were separated with the LSD procedure with a confidence level of 0.05 (Genstat 5 committee, 1993).

## Results

No feed refusals were observed during the collection period. The pigs fed the 10%-SBPS diet had a higher rate of gain than the pigs fed the other three diets, although the difference was not statistically significant (P > 0.05) (Table 3). Due to the higher nitrogen content of SBPS compared to tapioca, nitrogen intake differed slightly among diets. Increasing the level of SBPS to 10 and 15% in the diet increased nitrogen intake (P < 0.05). Dietary level of SBPS did not affect the nitrogen retention in pigs (P > 0.05). The apparent faecal nitrogen digestibility was negatively related to the level of SBPS in the diet. Apparent faecal nitrogen digestibility tended to decrease when dietary level of SBPS increased to 15% (P < 0.01). The amount of the fresh slurry produced by pigs was not influenced by the diet. However, increasing dietary level of SBPS increased slurry DM content (Table 4) and therefore increased total slurry DM production.

Table 4 shows the composition of slurry, sampled at the end of the collection period, from pigs fed different levels of SBPS in the diet. The diet did not affect the total N, ash and  $NH_4^+$ -N contents (P > 0.05). The level of SBPS in the diet strongly influenced the concentration of VFA (P < 0.001). Generally, the different VFA and the total VFA increased with increasing level of SBPS in the diet (P < 0.001). The following regression line was found between the amount of SBPS (in %) in the diet and the concentration of the total VFA (in g/kg) in the slurry:

$$TVFA = 9.33 (\pm 0.62) + 0.91 (\pm 0.07) \times SBPS$$
(1)

For each percentage increase of SBPS in the diet, the total VFA in the slurry was increased by 0.91 g/kg. The model explained for 94% of the variation in the total VFA.

Table 5 shows the pH measured in the slurry channel and in the laboratory set up of slurry from pigs fed different level of SBPS. The pH of slurry was measured directly in the channel, at the upper layer and a depth of 10 cm and after mixing the slurry, on day 7 of the collection period.

Variables	0%-SBPS	5%-SBPS	10%-SBPS	15%-SBPS	SEM	<b>P</b> <sup>1</sup>
Initial BW <sup>2</sup> , kg	44.76	44.79	45.59	45.79	1.006	NS
Final BW <sup>3</sup> , kg	57.82	59.58	60.97	59.56	1.081	NS
Weight gain <sup>4</sup> , g/d	654	634	704	652	26.60	NS
N intake, g/d Apparent N	42.31 <sup>a</sup>	44.23 <sup>ac</sup>	45.99 <sup>bc</sup>	45.77 <sup>6</sup>	0.710	*
digestibility, %	75.47ª	75.87 <sup>*</sup>	74.23ª	72.07 <sup>b</sup>	0.555	**
N retention, g/d	17.55	18.01	20.55	18.25	0.760	NS
Slurry amount, g/d	2466	2540	2457	2276	134	NS

Table 3. Animal BW gain, N intake, N retention, apparently faecal N digestibility
and slurry production of pigs fed different levels of sugar beet pulp silage

Probability of a significant treatment effect. \* P < 0.05; \*\* P < 0.01; NS = not significant.

<sup>2</sup>The pigs were weighed on day 1 of the 20-day experimental period.

<sup>3</sup>The pigs were weighed on day 20 of the 20-day experimental period.

<sup>4</sup>Calculated for 7 days of the collection period (week 3).

<sup>a,b,c</sup>Means within a row lacking a common superscript letter differ (P < 0.05)

In general the pH was strongly influenced by SBPS in the diet (P < 0.001). As dietary level of SBPS increased, the pH of slurry decreased. For each 5% increase in SBPS, the pH of the slurry reduced by 0.4 to 0.5 unit. The pH measured at a depth of 10 cm was on average 0.51 unit lower than the pH measured at the upper level. During the in vitro measuring period of ammonia emission, the pH followed the same pattern of those observed in the slurry channel. The pH of the slurry, measured at the upper, the middle and the bottom layers, were lowest for the pigs fed the 15%-SBPS diet and highest for the pigs fed 0%-SBPS diet. At similar level, the pH of slurry measured at the upper layer on day 7 of the collection period in the slurry pit was on average 0.28 unit lower than the pH of the slurry measured during the in vitro experiment when no fresh urine and faeces were added. Contrarily, the pH measured at the bottom level was on average 0.30 unit higher.

Components	0%-SBPS	5%-SBPS	10%-SBPS	15%-SBPS	SEM	P'
DM, g/kg	85.6ª	87.5ª	92.7 <sup>b</sup>	111.8 <sup>c</sup>	4.54	*
Ash, g/kg	25.2	24.8	25.0	28.4	1.17	NS
Total N, g/kg	6.28	6.71	6.52	7.15	0.39	NS
NH₄ <sup>+</sup> -N, g/kg	3.57	3.88	3.78	3.92	0.15	NS
VFA, g/kg						
Acetate	5.11ª	9.15 <sup>b</sup>	12.82 <sup>c</sup>	14.18 <sup>d</sup>	1.11	***
Propionate	1.80ª	1.61ª	2.28 <sup>a</sup>	3.40 <sup>b</sup>	0.24	**
Butyrate	0.77 <sup>a</sup>	1.67 <sup>ac</sup>	2.45 <sup>bc</sup>	3.28 <sup>b</sup>	0.40	*
Total VFA <sup>2</sup>	9.04ª	13.92 <sup>b</sup>	19.15°	$22.42^{d}$	0.75	***

<sup>1</sup>Probability of a significant treatment effect. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; NS = not significant.

<sup>2</sup>Acetate + Propionate + Butyrate + Isobutyrate + Valerate + Isovalerate

<sup>a,b,c,d</sup>Means within a row lacking a common superscript letter differ (P < 0.05)

Variables	0%-SBPS	5%-SBPS	10%-SBPS	15%-SBPS	SEM	$P^{I}$
pH in the pit						
Upper layer	8.81ª	8.42 <sup>b</sup>	7.77°	7.29 <sup>d</sup>	0.07	***
Bottom layer	8.37ª	7.82 <sup>b</sup>	7.23°	6.84 <sup>d</sup>	0.08	***
After mixing	8.32 <sup>a</sup>	7.88 <sup>b</sup>	7.38°	6.99 <sup>d</sup>	0.05	***
pH in vitro						
Upper layer	8.95 <sup>a</sup>	8.54 <sup>b</sup>	8.18 <sup>c</sup>	7.72 <sup>d</sup>	0.08	***
Middle layer	8.73ª	8.17 <sup>b</sup>	7.81°	7.45 <sup>d</sup>	0.11	***
Bottom layer	7.93ª	7.44 <sup>b</sup>	7.05°	6.66 <sup>d</sup>	0.07	***
Mean pH	8.54*	8.05 <sup>b</sup>	7.68°	7.28 <sup>d</sup>	0.05	***

Table 5. The pH of slurry, measured during 7 days, of pigs
fed different levels of sugar beet pulp silage

<sup>1</sup>Probability of a significant treatment effect: \*\*\* P < 0.001.

<sup>a,b,c,d</sup> Means within a row lacking a common superscript letter differ (P < 0.05)

The effects of dietary SBPS on the ammonia emission from slurry over 7 days are illustrated in Figure 1. Increasing the level of SBPS in the diet strongly decreased the ammonia emission (P < 0.001). The overall mean ammonia emission over the 7-day measuring period was 169.6 mmol. The mean ammonia emissions were 215.0, 188.5, 145.7 and 129.0 mmol for the 0%-SBPS, 5%-SBPS, 10%-SBPS and 15%-SBPS diets, respectively. On average, ammonia emission was reduced by 12, 32 and 40% when tapioca in the diet was exchanged by 5, 10 and 15% of SBPS, respectively. The following regression line was calculated between the ammonia emission (in mmol) from the slurry and the amount of SBPS (in %) in the diet:

Ammonia emission = 
$$214.6 (\pm 4.44) - 6.02 (\pm 0.47) \times SBPS$$
 (2)

For each percentage increase of SBPS in the diet, the ammonia emission from slurry was reduced by 6.02 mmol. The model explained for 93.6% of the variation in ammonia emission.

## Discussion

This experiment was designed to lower the pH and the ammonia emission from pig slurry by increasing the level of NSP by including SBPS in the diet, whilst maintaining a normal growth performance of animals. The results from this study support the concept that increasing dietary level of NSP leads to a reduction in the slurry pH, consequently, reduces the ammonia emission from the slurry. The levels of substrate used in this experiment ranged from 0 to 5, 10 and 15% of SBPS in the diets, therefore NSP contents increased from 27.5 to 30.46, 33.98 and 36.54%, respectively.

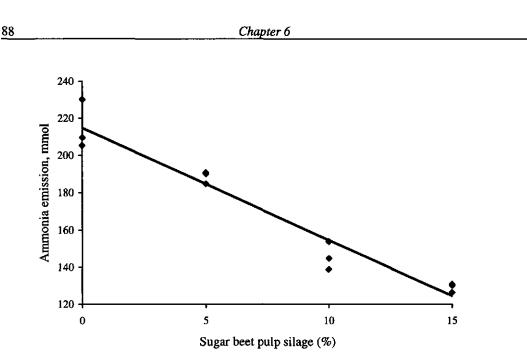


Figure 1. Relationship between ammonia emission from slurry and sugar beet pulp silage in the diet.

The results demonstrated that increasing the amount of NSP in the diet up to 36.5% did not influence performance of pigs. The slightly reduced apparent faecal nitrogen digestibility by pigs fed the high level of SBPS is in agreement with the findings of several researchers, e.g., Dierick *et al.* (1983), Longland and Low (1988), Canh *et al.* (1996). However, it is of importance to note that the digestibility of essential amino acids was not depressed by sugar beet pulp when measured at the end of the small intestine (Dierick *et al.*, 1983; Longland and Low, 1988). A high-NSP diet causes a shift of nitrogen from urine to faeces (Low, 1985; Morgan and Wittemore, 1988; Canh *et al.*, 1996 and 1997). This nitrogen is used for microbial protein synthesis in the hind gut of pigs and finally is excreted in the faeces. Thus despite the exchange of starch against NSP between the experimental diets, N retention was not affected by the diet.

Dietary SBPS strongly affected the pH of slurry. The pH of slurry was lower in pigs fed a high-NSP diet than in pigs' fed a low-NSP diet. According to Sommer and Husted (1995), VFA is an important factor influencing the pH of slurry. Volatile fatty acids are mainly formed by bacterial fermentation in the hind gut of pigs (Chabeauti *et al.*, 1991; Salvado *et al.*, 1993) and by anaerobic digestion of the slurry during storage (Spoelstra, 1979, Canh *et al.*, 1997). In pigs, VFA are mainly produced in faeces by microbial fermentation of dietary NSP and by deamination of amino acids. Only a non-significant amount of VFA originates from urine (Spoelstra, 1979; Chabeauti *et al.*, 1991; Salvado *et al.*, 1993). The rates of VFA production depend on the amount and characteristics of NSP. In a previous research, the effect of the amount and sources of fermentable NSP on the VFA and the pH of

#### Non-starch polisaccharide from sugar beet pulp silage and ammonia emission

slurry was evaluated. The results showed that cellulose and hemicellulose were positively related to the total amount of VFA in the slurry and the lignin content had a negative effect (Canh *et al.*, 1996 and 1997). According to Longland and Low (1988), Chabeauty *et al.* (1991) and Salvado *et al.* (1993), sugar beet pulp has a considerable content of NSP. The quality of NSP in sugar beet pulp is rather ideal for bacterial fermentation: low content of lignin and high content of pectin, hemicellulose and cellulose. In the present study the proportion of NSP was increased from 27.50 to 36.54% when SBPS increased from 0 to 15% in the diet. Results from this experiment show that large quantities of VFA were produced in the faeces of pigs and in the slurry during storage. A high NSP content in the diet enhanced VFA production.

In general, as dietary level of NSP increased the amount of each of the VFA and the total VFA in the slurry increased. Acetic acid was predominant in the VFA pool, followed by smaller amounts of propionic acid and butyric acid. These results are in agreement with the findings of Salvado *et al.* (1993) and Farnworth *et al.* (1995), who found acetic acid to be the most abundant VFA in the faecal and manure samples. The increased amount of VFA resulted in a lower pH of the slurry. This pH reduction was also persistent during the storage of the slurry in the in vitro measuring period of ammonia emission. The pH of the slurry at the upper level in the pit was lower than that observed in the laboratory system. This could be due to an effect of the daily addition of fresh faeces and urine to the surface of the slurry in the pit compared with the slurry stored in static conditions in the laboratory where no fresh faeces and urine was added.

As expected, the ammonia emission from the slurry was strongly affected by the level of SBPS in the diet. Increasing dietary NSP decreased the ammonia emission. According to Muck and Steenhuis (1981), ammonia is a conversion product of urea in the urine. Urea is converted into ammonia and carbon dioxide by the enzyme urease present in faeces. The  $NH_4^+$  concentration, temperature and the pH of the slurry are the most important factors influencing the ammonia emission. It was expected in this study that the NH4<sup>+</sup> concentration in the slurry would be lower when more SBPS was included in the diet, because of a shift in N excretion from urine to faeces. However, it seems that a little increase in nitrogen intake of pigs fed the higher SBPS diets did compensate for this shift. Ammonia losses from the slurry during the 7-day collection period in the climate chamber varied between diets and might also alter the NH4<sup>+</sup> concentration in the slurry at the end of the collection period. Ammonium concentrations of the slurry were not different between diets. Thus, in this study, at a fixed temperature, the decrease of the slurry pH was the main reason for the reduction of the ammonia emission when increasing the level of SBPS in the diet. On average the ammonia emission decreased with about 15% for each 5% increase of SBPS in the diet. However, the highest change in ammonia emission was obtained when the level of SBPS in the diet increased from 5 to 10%. The relatively low ammonium concentration of the slurry from the pigs fed 10%-SBPS was probably the main reason for this. Higher levels of SBPS might decrease the water intake, causing higher DM and  $NH_4^+$  concentrations, although the amount of slurry was not different between diets.

In this research, the ammonia emission was measured for a short time with an in vitro laboratory system. In commercial pig houses, the situation is different, mainly because of the dynamic situation in which continuously fresh urine and faeces are added to the slurry pit. The amount of VFA produced from fresh faeces may be obtained at higher levels. This increase of VFA can further affect the pH and ammonia emission from the slurry. Nevertheless, the potential impact of sugar beet pulp silage in the diet on ammonia emission has been demonstrated in this research.

# Conclusions

Increasing the level of non-starch polysaccharides by including more sugar beet pulp silage in the diet of growing-finishing pigs increases the concentration of volatile fatty acids in the slurry resulting in a lower pH of the slurry. This consequently, reduces the ammonia emission from slurry. This approach may be an economical way to reduce the ammonia emission from pig production system.

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# **Chapter 7**

# Influence of electrolyte balance and acidifying calcium salts in the diet of growing-finishing pigs on urinary pH, slurry pH and ammonia volatilisation from slurry

T.T. Canh<sup>a,b,c,\*</sup>, A.J.A. Aarnink<sup>a</sup>, Z. Mroz<sup>d</sup>, A. W. Jongbloed<sup>d</sup>, J. W. Schrama<sup>b</sup> and M.W.A. Verstegen<sup>b</sup>

<sup>a</sup>Department of Livestock Engineering, Institute of Agricultural and Environmental Engineering (IMAG-DLO), Wageningen, The Netherlands.

<sup>b</sup>Wageningen Institute of Animal Science (WIAS), Departments of Animal Nutrition and Animal Husbandry, Wageningen Agricultural University, The Netherlands.

<sup>c</sup>Netherlands Foundation for the Advancement of Tropical Research.

<sup>d</sup>Department Nutrition of Pigs and Poultry, Research Institute for Animal Science and Health (ID-DLO), The Netherlands.

\*Tel. (31) 317 476552; Fax: (31) 317 425670; E-mail: T.T.Canh@IMAG.DLO.NL

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# Influence of electrolyte balance and acidifying calcium salts in the diet of growing-finishing pigs on urinary pH, slurry pH and ammonia volatilisation from slurry

T.T. Canh, A.J.A. Aarnink, Z. Mroz, A. W. Jongbloed, J. W. Schrama and M.W.A. Verstegen

# Abstract

This study investigated the effects of dietary electrolyte balance (dEB = Na + K – Cl meq/kg DM) and acidifying Ca-salts on the pH and the ammonia emission from slurry of growing-finishing pigs. Ninety gilts of about 40 kg BW were randomly allotted to 18 diets used in five replicates. Two basal diets were used. Diet A had a high dEB (320 meq/kg DM) and diet B a low dEB (100 meq/kg DM). Each diet was supplemented with one of the four Ca-salts (CaCO<sub>3</sub>, CaSO<sub>4</sub>, Ca-benzoate or CaCl<sub>2</sub>) to increase the Ca content by 3 or 6 g per kg of diet. Faeces and urine were collected separately in metabolism cages and mixed as slurry. From a subsample of this slurry, pH and ammonia emissions were measured in a laboratory system. pH of urine and slurry, and the ammonia emission from the slurry, were lower at the low dEB level. Ammonia emission was reduced by 30, 33 and 54% when dietary CaCO<sub>3</sub> was replaced by CaCl<sub>2</sub>, CaSO<sub>4</sub> and Ca-benzoate, respectively. It is concluded that ammonia emission can be reduced by decreasing dEB level and adding CaSO<sub>4</sub> and CaCl<sub>2</sub> to the diet instead of CaCO<sub>3</sub>. The most profound effect is achieved when CaCO<sub>3</sub> is replaced by Ca-benzoate.

#### Keywords: Pig, Electrolytes, Ca-salts, pH, Ammonia

## Introduction

Ammonia emission from pig houses into the environment is a major problem in intensive pig production (Muck and Steenhuis, 1981; Apsimon *et al.*, 1995). Ammonia is a conversion product of urea in the urine catalysed by the enzyme urease present in faeces. Its emission into the air is a slow process governed by factors such as ammonia concentration, pH and temperature (Freney *et al.*, 1983; Aarnink, 1997). There are a number of ways to reduce ammonia emission by means of nutrition (Jongbloed and Lenis, 1992; Kreuzer and Machmüller, 1993; Kirchgessner *et al.*, 1994; Canh *et al.*, 1996).

The effect of pH on ammonia emission is very strong. Some researchers reduced the pH of slurry by acidification (Stevens et al., 1989; Hoeksma et al., 1993). However, lowering the pH of slurry by acidification may give other environmental problems. In previous research, Canh et al. (1997) found a

clear effect of dietary electrolyte balance (dEB = Na + K - Cl) on the pH of urine and slurry from growing-finishing pigs. The dietary cation-anion balance is an important factor affecting the acid-base status in animals. The maintenance of a constant blood pH is very critical for normal body function, and therefore, urinary acidity is the result of renal regulation of the acid-base balance in the body (Patience *et al.*, 1987; Tucker *et al.*, 1988; Haydon and West, 1990; Kienzle *et al.*, 1991; Kemme-Kroonsberg, 1993). Although there is evidence of the influence of dEB and acidifying salts on urinary pH, little information is available on their effects on the slurry pH and ammonia emission from pig slurry.

The objective of this research was to investigate the effects of dEB and acidifying Ca-salts (CaCO<sub>3</sub>, CaSO<sub>4</sub>, Ca-benzoate and CaCl<sub>2</sub>) on the pH of urine and slurry, and on ammonia emission from slurry of growing-finishing pigs.

## Material and methods

#### Animals

Ninety growing gilts (Yorkshire × [Dutch Landrace × Finish Landrace]) of 40 kg BW were randomly allotted to 18 diets. Each diet was used consecutively in five replicates. From 40 kg onwards the animals were kept in groups and were fed individually with the experimental diets. When the animals reached 55 kg of body weight, they were individually placed in metabolic cages in an environmentally controlled room. The sizes of the cages were 1.2 m 0.6 m (length × width) and were made of galvanised steel with wooden slats. The 14-day experimental period consisted of a 7-day adaptation period to allow the pigs to become accustomed to the cages and to their new diets, and a 7-day period during which urine and faeces were collected. Room temperature was kept at 20°C and relative humidity at about 55%.

#### Experimental design

The 18 diets were tested according to a completely randomised block design. Two levels of dEB (320 and 100 meq/kg DM), four types of Ca-salts (CaCO<sub>3</sub>, CaSO<sub>4</sub>, Ca-benzoate and CaCl<sub>2</sub>) and two levels of Ca-salts (7 and 10 g Ca/kg) were tested in a complete  $2 \times 4 \times 2$  factorial arrangement. In addition, two basal diets, containing 4 g Ca/kg, served as negative control diets (Table 2). The two basal diets (Table 1) were formulated to have equivalent amounts of NE, CP, and vitamins (ARC, 1981). To prevent the effect of dietary nitrogen on nitrogen excretion and subsequently on ammonia emission, the diets were made equal in ileal digestible essential amino acids by adding synthetic lysine, methionine, threonine and tryptophan.

Ingredients	Basal diet A	Basal diet B
(g/kg diet)	$dEB^{a)} = 320 \text{ meq/kg DM}$	dEB = 100 meq/kg DM
Maize	113.1	162.1
Barley	12.3	400.0
Tapioca	280.0	164.9
Extracted soyabean meal	196.6	_
Extracted coconut	-	34.6
Maize gluten feed	100.0	-
Maize gluten meal	-	100.0
Wheat middlings	149.4	61.9
Soyabean oil	41.0	-
Cane molasses	50.0	10.0
Maize starch	40.0	40.0
Limestone (CaCO <sub>3</sub> )	3.7	5.7
NaCl	3.0	3.0
Cr <sub>2</sub> O <sub>3</sub> -maize starch premix <sup>b)</sup>	1.0	1.0
Trace mineral-vitamin premix <sup>c)</sup>	1.0	1.0
Monocalcium phosphate	4.1	5.5
Choline chloride	0.3	0.3
L-Lysine. HCl	2.3	7.2
DL-Methionine	0.9	0.1
L-Threonine	1.1	1.9
L-Tryptophan	0.2	0.8
Calculated chemical composition		
(g/kg diet)		
DM	878.9	878.2
Ash	57.2	42.5
Total N	25.6	24.0
Ca	4.0	4.0
Mg	2.2	1.2
Total P	5.4	4.7
Na	1.8	1.3
K	11.6	6.4
Cl	3.5	4.2
Ileal dig. lysine <sup>d</sup>	8.0	8.0
Ileal dig. met+cyst <sup>d</sup> .	4.7	4.8
Ileal dig.threonine <sup>d</sup>	4.9	4.9
Ileal dig. tryptophan <sup>d</sup>	1.5	1.6
NE <sup>d</sup> (MJ/kg diet)	9.49	9.49

#### Table 1. Ingredients and chemical composition of basal diets (as-fed basis)

<sup>a</sup>dEB (dietary electrolyte balance) = Na + K - Cl (meq/kg DM).

<sup>b</sup>Cr<sub>2</sub>O<sub>3</sub> and maize starch mixed in proportion 1:3 (weight/weight).

<sup>c</sup>Contained the following ingredients (per kg of diet): 12.0 mg (6000 IU) vitamin A; 15.0 mg (1500 IU) vitamin D<sub>3</sub>; 10.0 mg (10 IU) vitamin E; 1.5 mg vitamin K<sub>3</sub>; 1.0 mg vitamin B<sub>1</sub>; 3.0 mg vitamin B<sub>2</sub>; 10.0 mg d-pantothenic acid; 15.0 mg niacin; 15.0  $\mu$ g vitamin B<sub>12</sub>; 1.0 mg vitamin B<sub>6</sub>; 250.0 mg FeSO<sub>4</sub>.7H<sub>2</sub>O (50 mg Fe); 62.8 mg CuSO<sub>4</sub> (25.0 mg Cu); 197.4 mg ZnSO<sub>4</sub>.7H<sub>2</sub>O (45.0 mg Zn); 33.8 mg of MnO (30.0 mg Mn); 0.7 mg CoSO<sub>4</sub>.7H<sub>2</sub>O (0.15 mg Co); 1.0 mg KI (0.8 mg I); 200.0  $\mu$ g of Na<sub>2</sub>SeO<sub>3</sub> (43.8  $\mu$ g Se); 380.4 mg maize starch as carrier.

<sup>d</sup>Taken from CVB (1994).

#### Chapter 7

		-			Diet				
Basal diet A, dEB <sup>a)</sup> = 320 meq/kg DM	1	2	3	4	5	6	7	8	9
Basal diet B, dEB = 100 meq/kg DM	10	11	12	13	14	15	16	17	18
Ca level (g/kg)	4	7	10	7	10	7	10	7	10
CaCO3 (g/kg)	-	8.8	17.6	-	-	-	-	-	-
CaSO <sub>4</sub> .2H <sub>2</sub> O (g/kg)	-	-	-	12	24	-	-	-	-
Ca-benzoate.2H2O (g/kg)	-	-	-	-	-	24	48	-	-
CaCl <sub>2</sub> .2H <sub>2</sub> O (g/kg)	-	-	-	-	-	-	-	11	22

Table 2. Experimental design

<sup>a</sup>dEB (dietary electrolyte balance) = Na + K - Cl (meq/kg DM).

They contained 320 meq dEB/kg DM (A) and 100 meq dEB/kg DM (B). The levels of dEB were different by adjustment of dietary ingredient compositions (tapioca meal and maize gluten feed or meal). Diet 2 was a control containing 7 g Ca/kg diet. Diets 1 and 10, containing 4 g Ca/kg diet, were designed as the negative controls for the high-dEB diets (from diets 2 to 9), and the low-dEB diets (from diets 11 to 18), respectively. CaCO<sub>3</sub>, CaSO<sub>4</sub>, Ca-benzoate and CaCl<sub>2</sub> were added to the other diets to increase the Ca content of the diets up to 7 and 10 g per kg. All added Ca-salts were supplied in substitution to maize starch (Tables 1 and 2). Because the dose of Ca-benzoate exceeded the amount of maize starch in the basal diets, tapioca was also partly substituted in diets 7 and 16. The pigs were fed 2.8 times the requirement for maintenance, which was assumed to be 418 MJ ME/BW<sup>0.75</sup> (ARC, 1981). One day before and after the collection period they were weighed and their rations were adjusted. The rations were increased each day on the basis of an estimated weight gain of 500 g per day. The feed was mixed with water (2.5 1 per kg of feed) and distributed in two equal meals. No additional water was given.

#### Measurements

Faeces and urine were collected and weighed twice daily. Urine was continuously collected via a balloon catheter fitted in the urinary bladder (Mroz *et al.*, 1996). Urine was voided in the urinary drainage bags (vacutainers) of 2 l with a tube of 90 cm, allowing the urine to be excreted under vacuum. The bags were replaced twice daily, in the morning and in the afternoon. The pH of the urine was measured twice a day directly after the collection (pH-meter, type CG 818, Schott-Geräte GmbH, Hofheim a. Ts., Germany). Daily samples of urine and faeces of each animal (20% of the total weight) were kept at -20°C for chemical analyses. The rest of the urine and faeces (80% of the total weight) were mixed as slurry. The slurry of each animal was kept in plastic buckets of 60 cm diameter. The buckets were placed in a room at 20°C. During each of the 7 days of the collection period, fresh urine and faeces were added to the buckets twice daily, immediately after the collection.

Each morning, the pH of the slurry was measured, before fresh urine and faeces were added, in the

top layer and 10 cm below. After adding fresh urine and faeces and a little mixing, the pH was measured again. After the 7-day collection period, the pooled slurry was mixed and sampled for chemical analyses and for in vitro ammonia emission measurements.

Ammonia emission was measured in a laboratory model at  $20^{\circ}$ C for seven days, following the procedures described by Derikx and Aarnink (1993). A sample of 2 kg of slurry per pig was placed in a vessel with an inside area of 284 cm<sup>2</sup> covered by a lid. Air entered the vessel by small holes in the edge of the lid and left the vessel in the centre. Ammonia in the outgoing air was removed by passing through two impingers, each containing 100 ml HNO<sub>3</sub> (0.5 M). The second impinger served as a control and contained no more than 5% of the amount of ammonia trapped in the first impinger. The air left the system after passing a water trap, a flow controller adjusted to 4.2 l/min and a pump. The first impinger was replaced after 1, 2, 4 and 7 days and the second impinger after 7 days. The ammonia concentration and the volume of the liquid were determined in the first and the second impingers. The cumulative ammonia emission was calculated by multiplying the volume by the ammonia concentration. The pH of the slurry was measured at the top, middle and bottom layers of the slurry at the same time when the first impinger was replaced.

## Chemical analyses

The diets were analysed in triplicate for CP, DM, ash, N, Ca, P, Mg, Na, K, Cl and buffer capacity (Mroz *et al.*, 1996). The Ca concentrations in all the Ca-salts were analysed in duplicate before these salts were added to the basal diets (Mroz *et al.*, 1996). Samples of slurry after the collection period were analysed in duplicate for DM, ash, N, and ammonium contents, according to AOAC (1994).

#### Statistical analyses

The data were analysed by ANOVA using the procedure of Genstat software (Genstat 5 Committee, 1993) for a  $2 \times 4 \times 2$  factorial treatment arrangement in a completely randomised design. Treatment effects were dEB, Ca-salt and Ca-salt level. The pig was the experimental unit. Trial was treated as a block. Slurry composition, pH of urine and slurry, and ammonia emission were the response variables in the following model:

$$Y_{ijkl} = \mu + B_i + E_j + S_k + L_l + (E \times S)_{jk} + (E \times L)_{jl} + (S \times L)_{kl} + (E \times S \times L)_{jkl} + e_{ijkl}$$
(1)

where Y = response variable;  $\mu$  = overall mean; B<sub>i</sub> = block effect (i = replicate 1 to 5); E<sub>j</sub> = effect of dEB (j = 320, 100 meq/kg DM); S<sub>k</sub> = effect of Ca-salt (k = CaCO<sub>3</sub>, CaSO<sub>4</sub>, Ca-benzoate, CaCl<sub>2</sub>); L<sub>1</sub> = effect of Ca-salt level (l = 7, 10 g Ca/kg); e<sub>ijkl</sub> = residual error; E × S; E × L; S × L and E × S × L = two and three-way interactions.

Additionally, the two negative control diets (Diets 1 and 10) containing 4 g Ca/kg diet were

compared with the diets containing 7 and 10 g Ca/kg feed for each dEB level. The standard errors of differences calculated with the model were used in the t-tests to calculate the least significant differences (LSD) between diets.

The effects of dietary electrolytes (in meq/kg DM):  $K^+ + Na^+$ ,  $CO_3^{2-}$ ,  $SO_4^{2-}$ ,  $CI^-$  and benzoate, on the pH of urine and slurry and on ammonia emission were evaluated using the REML model (Genstat 5 Committee, 1993). In this model the electrolytes were defined as fixed effects and blocks were defined as random effects. Because the sulphur concentrations of the basal diets were not analysed, they were assumed to be similar.

#### Results

#### Slurry amount and composition

There were no differences in the amounts of slurry between diets (Table 3). Generally, the DM content of slurry reduced when dEB was decreased. The DM content of slurry from pigs fed CaCO<sub>3</sub> and CaCl<sub>2</sub> were slightly lower than those observed on the other two Ca-salts. Decreasing the levels of dEB and Ca-salts in the diet reduced slurry ash content. Dietary electrolyte balance and Ca-salts did not affect total nitrogen content. With the exception of the diet with the high level of Ca-benzoate, differences in the ammonium content of slurry between the other diets were not significant.

### pH of urine and slurry

The pH of urine and slurry was strongly affected by dEB and acidifying Ca-salts (Table 4). In general, urinary pH of pigs fed the low-dEB diets was lower than of those fed the high-dEB diets. On average, it decreased by 0.46 unit when dEB decreased from 320 to 100 meq/kg DM. Including CaSO<sub>4</sub>, CaCl<sub>2</sub> and Ca-benzoate in the diet decreased urinary pH. The pH of urine from pigs fed the Ca-benzoate based diet was the lowest. No significant difference in urinary pH between the CaSO<sub>4</sub>- and CaCl<sub>2</sub>-based diets was found. The pH of urine was the highest on the CaCO<sub>3</sub>-based diet. It was 1.61, 1.66 and 1.80 unit higher than the pH of urine from the CaSO<sub>4</sub>-, CaCl<sub>2</sub>- and Ca-benzoate-based diets, respectively.

The pH of slurry measured during the collection period followed the same pattern as that observed for the urinary pH. On average, lowering dEB from 320 to 100 meq/kg DM reduced slurry pH by 0.17 unit. The pH of slurry was the lowest for the Ca-benzoate based diet, followed by the CaSO<sub>4</sub>- and CaCl<sub>2</sub>-based diets. It was the highest for the CaCO<sub>3</sub>-based diets. Increasing dietary levels of CaSO<sub>4</sub>, CaCl<sub>2</sub> and Ca-benzoate decreased the pH of slurry, whereas increasing level of CaCO<sub>3</sub> increased the pH of slurry. During the in vitro measuring period of ammonia emission, when no fresh urine and faeces were added, the pH of slurry was slightly lower than that measured during the collection period. But differences in the pH of slurry between diets were very similar to those observed during the collection period.

100

:					Type and	level of Ca-si	Type and level of Ca-salts (g /kg diet)	iet)		
Composition	co <sup>3</sup>	co <sup>3</sup> ;	co3	SO4 <sup>2-</sup>	SO4 <sup>2</sup>	Benzoate	Benzoate Benzoate	י ק	¢ CL	LSD <sup>b</sup>
	4	-	10	-	ĩ	~	0]	~	Π	
Amount (g/d.pig)										
dEB = 320  meq/kg DM	3803	3734	4012	3658	3757	3784	3769	3890	3815	442
dEB = 100  meq/kg DM	3683	3620	3760	3582	3825	3692	2508	3824	3884	
DM (g/kg)										
dEB = 320 meq/kg DM	83.0	82.2	80.6	92.3	101.5	0.06	91.2	85.7	78.3	11.5
dEB = 100 meq/kg DM	62.4	69.1	71.7	79.1	78.2	74.0	85.9	64.8	74.6	
Ash (g/kg)										
dEB = 320  meq/kg DM	22.5	23.7	24.8	26.4	30.3	23.3	21.7	25.3	23.8	2.7
dEB = 100  meq/kg DM	12.8	15.5	18.2	17.7	20.3	15.8	17.6	14.6	17.9	
N <sub>tot</sub> (g/kg)										
dEB = 320 meq/kg DM	6.48	6.48	6.14	6:39	7.09	6.40	5.53	6.36	6.28	0.70
dEB = 100 meq/kg DM	6.08	6.11	6.08	6.57	6.64	6.32	6.91	5.97	6.53	
NH4-N (g/kg)										
dEB = 320  meq/kg DM	3.89	3.92	3.81	3.55	3.88	3.40	2.25	3.75	3.83	0.60
dEB = 100  meq/kg DM	3.86	3.72	3.90	3.93	3.90	3.61	2.88	3.67	4.23	

Table 3. Amount and chemical composition of slurry from pigs fed experimental diets

101

Table 4. Mean pH<sup>a)</sup> of urine and slurry from pigs fed experimental diets

<sup>c</sup>Significant level of treatment factors: E: dEB; S: Ca-Salt; L: Ca-salt level; <sup>\*</sup>P < 0.05; <sup>\*\*</sup>P < 0.01; <sup>\*\*\*</sup>P < 0.001.</li>
<sup>d</sup>Dietary electrolyte balance = Na + K - Cl (meq/kg DM).
<sup>e</sup>During the 7-day collection period fresh urine and facces were daily added to a bucket with slurry.
<sup>f</sup>During the 7-day measuring period of ammonia emission no fresh urine and facces were added.

		Urinary pH	I		Slurry pH		
Electrolyte	βª	s.e <sup>b</sup>	P <sup>c</sup>	βª	s.e <sup>b</sup>	P <sup>c</sup>	
Constant	6.19	0.17	***	7.82	0.11	***	
K and Na	0.0020	0.0004	***	0.0006	0.0002	**	
CO3 <sup>2-</sup>	0.0031	0.0004	***	0.0017	0.0003	***	
SO4 <sup>2-</sup>	-0.0060	0.0004	***	-0.0030	0.0003	***	
CI.	-0.0069	0.0004	***	-0.0034	0.0003	***	
Benzoate	-0.0066	0.0004	***	-0.0058	0.0003	***	

 Table 5. Effect of different dietary electrolytes (in meq/kg DM) on the pH of urine and slurry

\*Coefficients.

<sup>b</sup>Standard error.

<sup>o</sup>Probability of significant effect: \*\*\*P < 0.001; \*\*P < 0.01.

The relationships between the different dietary electrolytes and the pH of urine and slurry were assessed (Table 5). Anion  $CO_3^{2-}$  and the sum of cations  $K^+$  and  $Na^+$  were positively related to urinary pH. The effects of the anions  $SO_4^{2-}$ , Cl<sup>-</sup> and benzoate were similar and negatively related to urinary pH.

The effects of different electrolytes on the average slurry pH were all clearly smaller than those estimated for urinary pH. However, the effect of benzoate was only slightly smaller than that estimated for the urinary pH. Therefore, the regression line was calculated without the data from the Ca-benzoate diets (Fig. 1) and was the following:

Slurry 
$$pH = 5.13 (\pm 0.19) + 0.42 (\pm 0.03) \times urine pH (R^2 = 0.73)$$
 (2)

#### Ammonia emission

The average ammonia emissions from slurry of pigs fed different diets, measured for 7 days, as a percentage of the ammonia emission from the positive control diet (CaCO<sub>3</sub>: 7g Ca/kg and dEB 320 meq) are illustrated in Figure 2. The overall mean cumulative ammonia emission for 7 days was 145.4 mmol. The dEB and acidifying Ca-salts strongly influenced ammonia emission. Reducing dEB from 320 to 100 meq/kg DM reduced ammonia emission by 11% (P < 0.01). Ammonia emission from slurry of pigs fed CaCO<sub>3</sub> was the highest, approximately about 30, 33 and 54% (P < 0.001) higher than those observed for the CaSO<sub>4</sub>-, CaCl<sub>2</sub>-, and Ca-benzoate-based diets, respectively. Including Ca-benzoate in the diets gave the lowest ammonia emission. There was no difference in ammonia emissions between the CaSO<sub>4</sub>- and CaCl<sub>2</sub>-based diets (P > 0.05). The largest effect of different electrolytes of the diet on ammonia emission (Table 6) was found for benzoate (Regression coefficient of -0.42) and the smallest for the sum of K<sup>+</sup> and Na<sup>+</sup> (Regression coefficient of 0.07).

Electrolyte	β³	s.e <sup>b</sup>	<i>P<sup>c</sup></i>
Constant	176	9	***
K and Na	0.07	0.02	***
CO <sub>3</sub> <sup>2</sup> SO <sub>4</sub> <sup>2</sup>	0.13	0.02	***
SO4 <sup>2-</sup>	-0.24	0.02	***
cr	-0.29	0.02	***
Benzoate	-0.42	0.02	***

Table 6. Effect of	different	dietary ele	ctrolytes (in	meq/kg DM) on
ammonia	emission	(in mmol/7	' days) from	slurry of pigs

<sup>b</sup>Standard error.

<sup>o</sup>Probability of a significant effect: \*\*\*P < 0.001

A positive relationship was found between the  $CO_3^{2-}$  content of the diet and ammonia emission. However, the absolute value of this coefficient was about half the values calculated for  $SO_4^{2-}$  and  $CI^-$ .

Figures 3 and 4 show the relationships between the natural logarithm (Log) of ammonia emission (in mmol/7 days) and the average pH of urine and slurry. The following regression lines were found:

Log (ammonia emission) = $3.76 (\pm 0.11) + 0.22 (\pm 0.02) \times urine pH$	$(R^2=0.84)$	(3)
Log (ammonia emission) = $1.40 (\pm 0.17) + 0.48 (\pm 0.03) \times slurry pH$	$(R^2 = 0.84)$	(4)

This means that when the pH of urine and slurry decreased by 1 unit, ammonia emission decreased by approximately 20 and 48%, respectively.

## Discussion

The results of this investigation demonstrate the effects of dEB and acidifying Ca-salts on the pH of urine and slurry and on ammonia emission from slurry of growing-finishing pigs. Ammonia emission is influenced by factors such as ammonia concentration, pH and temperature (Muck and Steenhuis, 1981; Freney *et al.*, 1983; Stevens *et al.*, 1989; Aarnink, 1997). In the present study, at a fixed temperature, the ammonium content of slurry was not different between diets, except for the diets with high level of Cabenzoate. The pH of slurry should, therefore, be the main factor causing the reduction of ammonia emission. This was confirmed by the high correlation coefficient between the pH of slurry and ammonia emission.

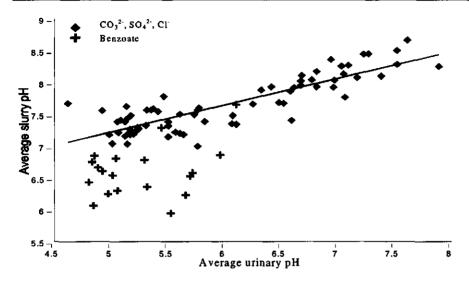


Figure 1. The pH of the slurry during the measuring period depends on the urinary pH. The regression line was calculated with the data of all diets except for the diets with Ca-benzoate. Slurry pH =  $5.13 (\pm 0.19) + 0.42 (\pm 0.03) \times$  urinary pH ( $R^2 = 0.73$ )

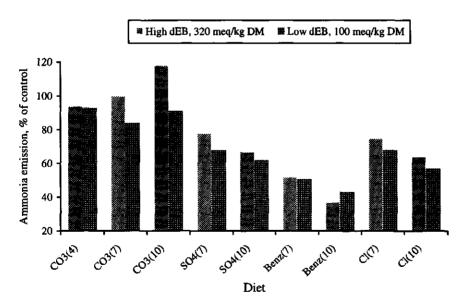


Figure 2. Ammonia emission from slurry of pigs fed different diets. Beneath the bars the supplemented Ca-salts with Ca levels in brackets. The control was the CaCO<sub>3</sub> diet with Ca level 7 g/kg and dEB 320 meq/kg DM.

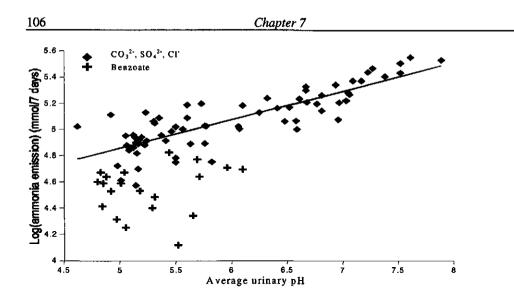


Figure 3. The logarithm of ammonia emission (in mmol/7 days) depending on the pH of the urine. The regression line was calculated with the data of all diets except for the diets with Ca-benzoate.  $Log(ammonia\ emission) = 3.76 (\pm 0.11) + 0.22 (\pm 0.02) \times urine\ pH (R^2 = 0.70)$ 

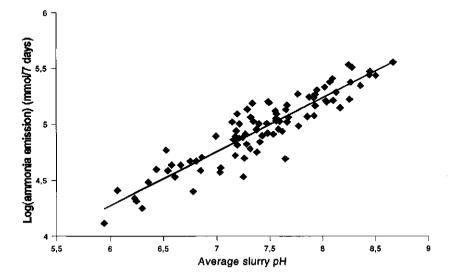


Figure 4. The logarithm of ammonia emission (in mmol/7 days) depending on the pH of slurry during the measuring period for all diets.  $Log(ammonia\ emission) = 1.40 (\pm 0.17) + 0.48 (\pm 0.03) \times slurry\ pH$  ( $R^2 = 0.84$ )

The lower ammonium concentrations of the slurry from pigs fed the high Ca-benzoate diets can be partly explained by the lower N intake caused by feed refusals of pigs fed these diets (Mroz *et al.*, 1996). Sommer and Husted (1995) found that, at a low pH of slurry, a complex is formed between magnesium, ammonium and phosphate. The low pH of the slurry from pigs fed the high Ca-benzoate diets might affect the formation of this complex, and therefore, lowering the ammonium concentration of slurry.

A general decrease in the pH of urine and slurry and in ammonia emission was observed for the low-dEB diets. In this study, dEB levels were different by adjustment of the ingredient compositions of the diets. This caused differences in dietary Na, K and Cl. In theory, any alteration of the relative amounts of Na, K and Cl in the diet will affect the pH of urine (Tucker *et al.*, 1988). Patience *et al.*(1987) reported that increasing dietary Na<sup>+</sup> and K<sup>+</sup> increases the pH of urine, whereas increasing dietary Cl<sup>-</sup> decreases the pH of urine. Witting and Cole (1986) found an increased net acid excretion in urine when replacing dietary carbonate by chloride. Rose (1989) showed that an excess of dietary K (hyperkalemia) reduces net acid secretion and a depletion of K (hypokalemia) increases net acid excretion.

It should be noted that other components in the basal diets might also have influenced slurry pH and ammonia emission. However, in this study the effect of dEB on urinary pH, causing a lower pH of slurry and a lower ammonia emission, seems to be the main effect.

The type of acidifying Ca-salts influenced slurry pH and ammonia emission. An increase in dietary CaCO<sub>3</sub> increased the pH of urine and slurry, resulting in a higher ammonia emission. In contrast, an increase in dietary CaSO<sub>4</sub>, CaCl<sub>2</sub> or Ca-benzoate reduced the pH of urine and slurry, thereby lowering ammonia emission. In this experiment Ca-salts were chosen to manipulate the dEB. This was done because Ca-salts are the most common salts in practical diets. Under usual conditions, Ca is primarily involved in skeletal development rather than in the acid-base balance (Mongin, 1981). It appears that the anions  $CO_3^{2^\circ}$ ,  $SO_4^{2^\circ}$ , Cl<sup>-</sup> and benzoate mainly affect the acid-base homeostasis. The observed effects of Ca-salts on urinary pH are consistent with other publications. In cats, Kienzle *et al.* (1991) found that the pH of urine increased with a high CaCO<sub>3</sub> and decreased with a high CaCl<sub>2</sub> diets. Haydon and West (1990) reported that decreasing dEB by substitution of CaCO<sub>3</sub> for CaCl<sub>2</sub> decreased the pH, base excess and HCO<sub>3</sub> of the blood. Consequently, these changes resulted in a reduction of urinary and slurry pH.

The addition of CaSO<sub>4</sub> to the diet had similar effects on pH and ammonia emission when CaCl<sub>2</sub> was added. This is in agreement with the expectation that the process in renal excretion of  $SO_4^{2^2}$  is similar to that of Cl<sup>-</sup>, because Cl<sup>-</sup>,  $SO_4^{2^-}$  are well absorbable acidifying agents. The absorbed  $SO_4^{2^2}$  is almost completely excreted by the kidney due to poor reabsorbability. Renal excretion of  $SO_4^{2^2}$  is accompanied by excretion of H<sup>+</sup>. This excretion of H<sup>+</sup> increases the net acid excretion, and therefore reduces the pH of urine. In Fig. 5, the mechanism of the acidifying properties of CaCl<sub>2</sub> is given schematically in a very simplified way. The main cause of the net acidifying effect of CaCl<sub>2</sub> or CaSO<sub>4</sub> is that the anions Cl<sup>-</sup> and  $SO_4^{2^2}$  are absorbed in the intestine to a greater extent than Ca<sup>2+</sup>.

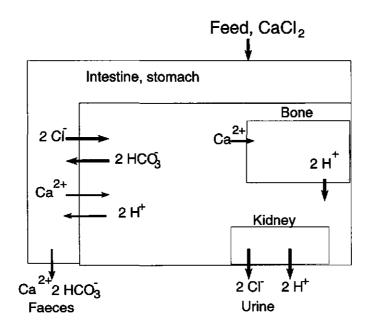


Figure 5. A simplified scheme of the electrolyte balance of a pig when fed CaCl<sub>2</sub>.

Furthermore, more  $Ca^{2+}$  is retained in the body, especially in the skeleton.  $H_2CO_3$  and  $H_3PO_4$  play a major intermediary role in the whole process of transportation and buffering (Kemme-Kroonsberg, 1993).  $H_2CO_3$  is a very weak acid and it is also easily converted to  $H_2O$  and  $CO_2$ . That explains the opposite effect of CaCO<sub>3</sub> on urinary and slurry pH when compared to CaSO<sub>4</sub> and CaCl<sub>2</sub>.

The most profound effect on slurry pH and ammonia emission from the slurry was found when Cabenzoate was added to the diet. This is in agreement with previous results of Mroz *et al.* (1997), who reported that adding Ca-benzoate to the diet lowered urinary pH from 7.25 to 5.66. The low urinary pH is a result of urinary excretion of hippuric acid, an end product of benzoate metabolism. In the liver, benzoate is conjugated with glycine to form hippuric acid or benzoylglucuronic acid, which are rapidly excreted in urine (Mroz *et al.*, 1996, 1997). Hippuric acid is a weak acid and increases the buffer capacity of the urine. This was confirmed by Mroz *et al.* (1996), who found a higher buffer capacity of the urine from pigs fed diets with Ca-benzoate than from pigs fed diets with CaCl<sub>2</sub> or CaSO<sub>4</sub>. This may explain the lower slurry pH for the benzoate diets than for the other diets, although urinary pH was similar.

Elzing and Aarnink (1996) estimated the effect of urinary pH on ammonia emission in a model situation of a pig house. They found a reduction of about 12% of ammonia release from the slurry pit when urinary pH decreased from 7 to 6. This effect is lower than that found under in vitro conditions

(about 20%, excluding the benzoate diets). The main cause could be that the different dietary factors not only influenced the pH and buffer capacity of the urine, but also of the faeces. The latter, however, was not measured in this experiment.

Practical diets contain about 320 meq of dEB/kg DM and a Ca-level of about 7 g/kg diet achieved by supplying CaCO<sub>3</sub>. This means that diet 2 resembles a practical diet. By adding CaSO<sub>4</sub> or CaCl<sub>2</sub> to the diet instead of CaCO<sub>3</sub> and reducing the dEB to 100 meq/kg DM, the urinary pH can be lowered by nearly 2 units. Ammonia emission from slurry would then be reduced by about 35%. Supplying Ca-benzoate instead of CaCO<sub>3</sub> would reduce ammonia emission from the slurry by about 48%. However, feed intake also slightly decreased at the high level of ca-benzoate in the diet.

In this research, ammonia emission was measured for a short period of time with an in vitro system. In commercial pig houses, the situation is different, mainly because of the dynamic situations in which continuous fresh urine and faeces are added to the slurry pit. Ad libitum water supply in commercial pig houses may result in a different water intake and thereby may influence slurry characteristics and ammonia emission.

Further research is necessary to validate the results found in this study under practical conditions, especially with respect to animal performance. Nevertheless, the potential impact of slurry characteristics derived from dEB and acidifying salts on ammonia emission has been demonstrated in this research.

# Conclusion

Altering the dietary electrolyte balance (Na + K - Cl) from 320 to 100 meq/kg DM generally reduces pH of the urine and slurry, therefore lowering the ammonia emissions from the slurry. The pH of urine and slurry and the ammonia emission from the slurry can be reduced considerably when the Casalts CaSO<sub>4</sub> or CaCl<sub>2</sub> are added to the diet instead of CaCO<sub>3</sub>. The most profound effect is achieved when CaCO<sub>3</sub> is replaced by Ca-benzoate. However, further research is necessary to verify these effects of dietary electrolyte balance and acidifying Ca-salts, especially of Ca-benzoate, on growth performance of pigs.

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# **Chapter 8**

# Dietary Protein Affects Nitrogen Excretion and Ammonia Emission from Slurry of Growing-Finishing Pigs

T. T. Canh<sup>a,b,c,\*</sup>, A.J.A. Aarnink<sup>a</sup>, J. B. Schutte<sup>d</sup>, A. L. Sutton<sup>e</sup>, D. J. Langhout<sup>d</sup>, M.W.A. Verstegen<sup>b</sup> and J. W. Schrama<sup>b</sup>

<sup>a</sup>Department of Livestock Engineering, Institute of Agricultural and Environmental Engineering (IMAG-DLO), Wageningen, The Netherlands.

<sup>b</sup>Wageningen Institute of Animal Science (WIAS), Departments of Animal Nutrition and Animal Husbandry, Wageningen Agricultural University, The Netherlands.

<sup>c</sup>Netherlands Foundation for the Advancement of Tropical Research.

<sup>d</sup>Institute of Animal Nutrition and Meat Technology (ILOB-TNO), Wageningen, The Netherlands <sup>e</sup>Department of Animal Sciences, Purdue University, USA

\*Tel. (31) 317 476552; Fax: (31) 317 425670; E-mail: T.T.Canh@IMAG.DLO.NL

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# Dietary Protein Affects Nitrogen Excretion and Ammonia Emission from Slurry of Growing-Finishing Pigs

T. T. Canh, A.J.A. Aarnink, J. B. Schutte, A. L. Sutton, D. J. Langhout, M.W.A. Verstegen and J. W. Schrama

#### Abstract

The effects of dietary protein on nitrogen excretion and ammonia emission from slurry of growing-finishing pigs were studied both in vitro and in a pig house. The three diets had similar contents of NE, minerals, vitamins and ileal digestible lysine, methionine + cystine, threonine and tryptophan, but differed in CP content (16.5, 14.5 and 12.5%).

In the balance experiment, 18 castrated males of about 55 kg BW were allotted to the three diets. The experiment lasted 9 weeks, which was divided into 3 periods. In each period, urine and faeces were collected separately for 7 days in metabolism cages and mixed as slurry. A sample of this slurry was placed in a laboratory system to measure ammonia emission for seven days. In the barn experiment, 216 pigs were housed in three compartments and fed the experimental diets. Ammonia emission was measured directly from the compartments for 7 days during each of the 3-week periods.

There was no effect of dietary CP level on faecal nitrogen excretion. Urinary nitrogen excretion and slurry pH decreased when dietary CP decreased. Both balance and barn experiments showed similar effects of dietary CP on ammonia emission from slurry. Ammonia emission was reduced by 10-12.5% for each percent decrease in dietary CP. It is concluded that lowering dietary CP and supplementing essential amino acids while maintaining normal growth rate reduces urinary nitrogen and ammonia emission from the slurry of growing-finishing pigs

#### Keywords: Pigs, Diet, Nitrogen, Slurry, Ammonia Emission

#### Introduction

Ammonia emission from intensive pig production systems contributes appreciably to environmental pollution (Apsimon and Kruse-Plass, 1990). Recently, feeding manipulation has been considered as an economical way to reduce ammonia emission from pig houses (Heinrichs and Oldenburg, 1993; Canh et al., 1997a, b; Sutton et al., 1997). Jongbloed and Lenis (1992) reported that about 20% of the total

ingested nitrogen in pigs are excreted in faeces and about 50% in urine. Nitrogen excreted via faeces is predominantly incorporated in bacterial protein, which is less susceptible to rapid decomposition. Nitrogen excreted via urine is mainly in the form of urea, which is easily converted into ammonia and carbon dioxide by the enzyme urease present in faeces.

There are two basic ways to reduce nitrogen excretion in pig urine: 1) by shifting nitrogen excretion from urine to faeces by increasing fibrous feedstuffs in the diet (Schulze et al., 1993; Canh et al., 1997a); 2) by reducing the nitrogen content of the diet (Spieker, 1992; Dourmad et al., 1992; Kay and Lee, 1997). However, reduction of dietary nitrogen content must be handled carefully in order to maintain normal animal performance. Essential amino acids must be supplied in accordance with pig requirements.

Although some research has been done to estimate the effect of dietary protein on ammonia emission (Latimier and Dourmad, 1993; Oldenburg and Heinrichs, 1996; Cole et al, 1996), there is little documented information in which the actual effect of dietary protein on ammonia emission from pig slurry was quantified. Therefore, in vitro and barn assays were conducted in parallel for a direct measurement of ammonia emission from the slurry of growing-finishing pigs.

#### Materials and methods

#### Diets and feeding

Three diets were formulated, based on the same level of wheat and barley as basic energy sources (Table 1). They had similar contents of NE, minerals and vitamins, but differed in crude protein level by the amount of soybean meal. Other factors such as dietary electrolyte balance (dEB) and fibrous components, which could possibly affect the pH and ammonium contents of slurry (Canh et al., 1997a, b), were composed at similar levels (Table 2). Diet 1 had the highest crude protein content (16.5%), followed by Diet 2 (14.5%) and Diet 3 (12.5%) (Table 2). The diets were made equal for the contents of ileal digestible lysine, methionine + cystine, threonine and tryptophan. The pigs were fed three times the energy required for maintenance, which was assumed to be 294 kJ NE/kg of metabolic weight BW<sup>0.75</sup> (ARC, 1981). The rations were increased each day on the basis of an estimated weight gain of 800 g per day. The feed was mixed with water (3.1 1 per kg of feed) and distributed in two equal daily meals. No additional water was given.

#### Balance experiment

Eighteen growing crossbred castrated male pigs [(Dutch Landrace × Great Yorkshire) × Great Yorkshire], weighing initially 55 kg, were randomly allotted to three experimental diets. The pigs were housed individually in metabolism cages in an environmentally controlled room. The temperature was kept at 20 °C and the relative humidity at about 65%.

Ingredient (g/kg diet)	Diet				
	16.5% CP	14.5% CP	12.5% CP		
Barley	37.50	37.50	37.50		
Wheat	37.50	37.50	37.50		
Tapioca	0.66	5.48	10.30		
Soybean oil	1.00	0.70	0.40		
Soybean meal(46.5% CP)	16.30	11.20	6.10		
Cane molasses	4.00	4.00	4.00		
Premix <sup>a</sup>	1.00	1.00	1.00		
NaCl	0.30	0.24	0.18		
Limestone(CaCO <sub>3</sub> )	1.10	1.07	1.04		
Monocalcium phosphate	0.45	0.50	0.55		
NaHCO <sub>3</sub>	0.05	0.13	0.21		
KHCO3		0.20	0.41		
L-Lysine HCl	0.11	0.28	0.45		
DL-Methionine	-	0.06	0.12		
L-Threonine	0.03	0.11	0.19		
L-Tryptophan	-	0.03	0.05		

#### Table 1. Ingredient composition of the experimental diets (as fed basic)

<sup>a</sup>The vitamin and mineral premix supplied per kg of the diet: 5.000 IU of vitamin A, 1.000 IU of vitamin D<sub>3</sub>, 200 mg of cholin-chlorid, 7.5 mg of vitamin E, 3.5 mg of riboflavin, 20 mg of niacinamid, 5 mg of d-panthothenic acid, 15 µg of vitamin B<sub>12</sub>, 0.4 mg of vitamin K, 125 mg of ZnSO<sub>4</sub>.H<sub>2</sub>O, 40 mg of MnO<sub>2</sub>, 400 mg of FeSO<sub>4</sub>.7H<sub>2</sub>O, 1 mg of CoSO<sub>4</sub>.7H<sub>2</sub>O, 0.2 mg of Na<sub>2</sub>SeO<sub>3</sub>.5H<sub>2</sub>O, 0.5 mg of KI, 80 mg of CuSO<sub>4</sub>.5H<sub>2</sub>O and 20 mg of virginiamycin.

The cages were made of galvanized steel with wooden slats. Their sizes were  $1.2 \times 0.6$  m (length  $\times$  width). The total experimental period lasted 9 weeks. During each 3-week sub-periods, urine and faeces were collected separately twice daily for 7 days (in weeks 3, 6 and 9). The urine and faeces from each pig collected during the first 4 days of each 7-d collection period were stored at -20 ° C until nitrogen balance analyses were performed. To prevent nitrogen losses by evaporation of ammonia, the pH of urine was kept below pH 2 by collecting the urine in 50 ml of 25% sulphuric acid. Urine and faeces collected during the last 3 days, without adding acid to the urine, were used to make the slurry. The urine and faeces from randomly selected pairs of pigs on the same diet were pooled and put in a plastic open bucket of 60 cm diameter. The buckets were placed in a room at 20 ° C. During 3 days of the collection period fresh urine and faeces were added twice daily to the buckets immediately after collection. After the 7-d collection period, the pooled slurry was mixed and sampled for chemical analyses and for measurement of in vitro ammonia emission.

		Diet	
Composition, %	16.5% CP	14.5% CP <sup>a</sup>	12.5% CP <sup>a</sup>
(as fed basic)			
CP	16.5	14.5	12.5
Ileal digestible CP	13.0	11.3	9.6
NE (kcal/kg)	2245	2245	2245
Dry matter	86.4	86.5	86.6
Crude fat	2.8	2.4	2.0
Crude fibre	3.3	3.3	3.3
Ash	4.8	4.8	4.8
Soluble carbohydrates	59.1	61.3	63.5
Starch	43.2	46.1	48.9
Sugar	5.0	4.6	4.2
Nonstarch Polysaccharides <sup>b</sup>	8.2	7.7	7.3
Ca	0.62	0.62	0.62
P	0.47	0.46	0.44
- Dig.P	0.20	0.20	0.20
Na	0.15	0.15	0.15
K	0.84	0.84	0.84
Cl	0.32	0.32	0.32
Mg	0.14	0.13	0.12
Linoleic acid	1.20	1.00	0.80
dEB <sup>c</sup> , mEq/100g	19.05	19.05	19.05
Amino Acids			
Lysine	0.85	0.84	0.82
Methionine	0.26	0.28	0.31
Meth.+ Cyst.	0.58	0.57	0.55
Threonine	0.61	0.59	0.58
Tryptofan	0.20	0.20	0.20
Isoleucine	0.66	0.56	0.45
Leucine	1.18	1.00	0.83
Valine	0.76	0.66	0.55
Histidine	0.38	0.32	0.26
Phenylalanine	0.79	0.68	0.56
Tyrosine	0.54	0.46	0.38
Phenyl.+ tyrosine	1.33	1.14	0.94
Ileal digestible amino acids			
Lysine	0.71	0.71	0.71
Methionine	0.22	0.25	0.28
Meth.+ Cyst.	0.46	0.46	0.46
Threonine	0.46	0.46	0.46
Tryptofan	0.16	0.16	0.16

#### Table 2. Chemical composition of the experimental diets

<sup>a</sup>Crude protein are included additional amino acids. <sup>b</sup>Nonstarch polysaccharides, determined as: Organic matter - (crude protein + crude fat + starch + sugar).

<sup>c</sup>Dietary electrolyte balance (dEB; calculated as mEq Na + K - Cl).

Ammonia emission was determined in vitro in a laboratory set-up at 20 °C for 7 days, following the procedures described by Derikx and Aarnink (1993). A sample of 2 kg slurry was placed in a vessel with an inside area of 284 cm<sup>2</sup> covered by a lid. A total of 9 vessels were used, 3 per diet. Air entered the vessel through small holes at the edge of the lid and left the vessel in the centre. Ammonia in the outgoing air was removed by passing through two impingers, each containing 100 ml HNO<sub>3</sub> (0.5 M). The second impinger served as a control and contained no more than 5% of the amount of ammonia trapped in the first impinger. The air left the system after passing a water trap, an air flow controller adjusted to 4.2 l/min and a pump. The first impinger was replaced after 3 and 7 days, and the second impingers were determined. The cumulative ammonia emission was calculated by multiplying the volume by ammonia concentration. The pH of the slurry was measured at the bottom, the middle and the top layers of the slurry at the same time the first impinger was replaced.

#### Barn experiment

To validate the results of the balance experiment under dynamic situations in pig houses, a total of 216 male and female pigs, of the same breed, similar age and BW as in the balance experiment, were housed in groups in three similar compartments, one for each diet. Each compartment had 12 pens of 6 pigs in each. Each pen consisted of two slatted metal floors, one at the front and the other at the back, separated by a domed solid floor. There was a slurry channel underneath each slatted floor. The sizes of the front slurry channel were  $12 \times 0.65 \times 0.5$  m, and of the back channel  $12 \times 1.5 \times 0.5$  m (length × width × depth). This experiment lasted also 9 weeks and was divided into three 3-week sub-periods. At the start of each sub-period after cleaning the slurry channels were filled with water to a depth of 5 cm. This was done to prevent air and urine leaching through the piping system and to facilitate mixing urine with faeces in the slurry channel. The incoming air was brought into the compartment through a duct underneath the feeding passage and then diffused out through the overlying slatted floor. The outgoing air was removed from each compartment through two ventilators located on either side of the ceiling. The temperature in the compartments was kept at 20  $^{\circ}$ C and the humidity at about 65%. Ventilation rate was maintained at about 55 m<sup>3</sup>/h.pig.

The pigs were weighed on the first day of each sub-period and their rations were adjusted. After each 3-week sub-period, the slurry from all pens of each compartment was mixed and sampled for chemical analyses. The pigs were exchanged between compartments but remained on the same diet. Compartments and slurry channels were then cleaned with water.

Ammonia emission from the compartments was measured during the last 7 days of each of the 3-week sub-period according to the methods described by Scholtens (1990) and Ouwerkerk (1993). Ammonia concentration in the incoming air was measured in the air duct, before it entered the compartment. The incoming and outgoing air from the compartments was continuously sampled. The air was conducted through heated teflon tubes to an ammonia convertor located outside the chamber,

where ammonia was converted into nitrogen oxide at 775 °C. The air then flowed from the convertor through heated teflon tubes to a  $NO_x$  analyser (model ML 8840, Monitor labs), where nitrogen oxide concentration was determined on the principle of chemiluminescence. Before each measuring period the monitor was calibrated with a gas of 40 ppm NO in N<sub>2</sub> and the flow in the channels was checked. The value measured with the analyser differed from the concentration of the standard gas by less than 5%. The filters to prevent dust from entering the measuring equipment were changed at the beginning of each 3-week sub-periods. The efficiency of ammonia conversion to nitrogen oxide by the convertors was about 90%.

The area fouled with urine was assessed visually twice daily before morning and afternoon feeds. A map of the fouled area was drawn on paper and the fouled area was calculated from the area fouled with urine relative to the total pen floor area.

The pH of slurry in the slurry channel was measured 4 times in each measuring period after morning feeding, in the top layer and at a depth of 10 cm. The pH at each level was calculated as the mean pH measured in 6 pens.

#### Chemical analysis

All samples were analysed in triplicate. Diets and slurry were analysed for DM, ash, and total-N according to AOAC (1990) procedures. Ammoniacal nitrogen  $(NH_4^+ + NH_3)$  in slurry was determined titrimetrically according to NEN 3235 (NEN, 1983). Data on NE, dEB, minerals and amino acids were obtained from CVB (1994), in which data on nutrient composition, digestibility and feeding values of feedstuffs have been presented along with the references for determining chemical composition. The pH of slurry was measured at room temperature with a Sentron instrument glass electrode (model 1001) directly submerged in the slurry.

#### Statistical Analysis

In the balance experiment, the pig was the experimental unit. The effects of dietary crude protein level on average daily gain, N intake, N retention, N excretion, apparent faecal N digestibility, and slurry composition, and on the pH and ammonia emission from slurry were tested for the mean of three periods using one-way ANOVA procedures (Genstat 5 committee, 1993). When diet effect was significant (P < 0.05), the means were compared with the LSD procedure using 0.05 confidence level. In the barn experiment, pen was experimental unit. Data on animal performance was tested also for the mean of three periods using one-way ANOVA procedures (Genstat 5 committee, 1993). Because only one value of slurry composition and ammonia emission was available for each diet, no statistical analysis was possible. Linear regression was used to evaluate the relationship between ammonia emissions measured in balance and barn experiments.

120

Variables		Diet			
	16.5% CP (6) <sup>a</sup>			. Р <sup>ь</sup>	SEM
Initial BW <sup>c</sup> (kg)	54.83	54.93	54.83	NS	1.04
Final BW <sup>d</sup> (kg)	105.6	107.3	105.7	NS	2.17
Weight gain <sup>e</sup> (g/d)	793	819	795	NS	28
Feed intake (g/d)	2361	2341	2334	NS	75
N intake (g/d)	61.51 <sup>f</sup>	54.41 <sup>g</sup>	46.50 <sup>h</sup>	***	1.74
Faeces (g/d)	934	935	911	NS	39.70
Faecal N (g/kg)	9.31	9.24	9.18	NS	0.23
Faecal N (g/d)	8.61	8.59	8.31	NS	0.33
Apparent N digestibility (%)	85.84 <sup>f</sup>	84.03 <sup>8</sup>	81.92 <sup>h</sup>	***	0.70
Urine (g/d)	3569	3500	3444	NS	138
Urine N (g/kg)	8.09 <sup>f</sup>	6.47 <sup>8</sup>	4.59 <sup>h</sup>	***	0.27
Urine N (g/d)	29.30 <sup>r</sup>	23.23 <sup>g</sup>	16.20 <sup>h</sup>	***	1.75
Total N excretion (g/d)	37.91 <sup>f</sup>	31.82 <sup>g</sup>	24.51 <sup>h</sup>	***	1.84
Urinary N:faecal N	3.40 <sup>r</sup>	2.79 <sup>g</sup>	2.00 <sup>h</sup>	***	0.30
N retention (g/d)	23.60	22.61	21.96	NS	0.72
N retention (% of intake)	39.10 <sup>r</sup>	42.23 <sup>f</sup>	47.90 <sup>g</sup>	**	1.78
N retention (% of dig.N)	45.60 <sup>f</sup>	50.40 <sup>f</sup>	58.60 <sup>8</sup>	**	2.26

Table 3. Weight gain, nitrogen intake, retention, excretion, and apparent faecal	
nitrogen digestibility in pigs fed different diets in the balance experiment	t

<sup>a</sup>Number of observations in parentheses.

<sup>b</sup>Probability of a significant treatment effect. P < 0.05; P < 0.01; P < 0.001 NS = not significant

<sup>c</sup>The pigs were weighed on day 1 of the 64-d experimental period.

<sup>d</sup>The pigs were weighed on day 64 of the 64-d experimental period.

Calculated for a period of 64 days between the initial BW and the final BW.

<sup>f.g.h</sup>Mean within a row lacking a common superscript letter differ (P < 0.05).

#### Results

#### Balance experiment

No health problems occurred during the experimental period, and no feed refusals were observed. The level of dietary CP (Table 3) did not influence feed intake and daily gain. Nitrogen intake decreased when dietary CP decreased (P < 0.05). Faeces and urine output did not differ between diets. Dietary CP level did not affect faecal nitrogen excretion (P > 0.05). On average, urinary nitrogen excretion was reduced by about 45% by dietary CP reduction from 16.5 to 12.5% (P < 0.001). As a result, the ratio of urinary nitrogen to faecal nitrogen decreased considerably when dietary CP decreased. Apparent faecal nitrogen digestibility decreased (P < 0.05) and nitrogen retention increased when dietary CP decreased.

Variables		Diet			
	16.5% CP	14.5% CP	12.5% CP	P <sup>c</sup>	SEM
DM (g/kg)					
Balance Exp.	81.24	74.10	75.29	NS	2.02
Barn Exp.	46.33	42.73	43.63		
Total N (g/100g DM)					
Balance Exp.	11.13 <sup>d</sup>	9.57°	7.65 <sup>f</sup>	***	0.56
Barn Exp.	9.84	8.80	7.22		
Ammoniacal N (g/100g DM)					
Balance Exp.	8.83 <sup>d</sup>	7.22 <sup>e</sup>	5.43 <sup>f</sup>	***	0.55
Barn Exp.	6.78	6.11	4.49		
Ash (g/100g DM)					
Balance Exp.	25.46	26.38	26.29	NS	0.62
Barn Exp.	25.02	25.50	26.03		
рН					
Balance Exp.	9.14 <sup>d</sup>	8.70 <sup>e</sup>	8.16 <sup>f</sup>	***	0.08
Barn Exp.	7.87	7.55	7.21		

#### Table 4. The pH and composition of slurry from pigs fed different diets in the balance<sup>\*</sup> and barn<sup>b</sup> experiments

<sup>a</sup>Slurry was sampled after the last 3 days of the collection period.

<sup>b</sup>Slurry was sampled after each 3-week sub-period.

<sup>c</sup>Probability of a significant treatment effect. P < 0.05; \*P < 0.01; \*\*P < 0.001 NS = not significant.

<sup>d,e,f</sup>Mean within a row lacking a common superscript letter differ (P < 0.05).

The concentrations of DM and ash in slurry (Table 4) were not different between diets. Dietary CP significantly influenced total nitrogen and ammoniacal nitrogen concentrations, and slurry pH. On average, reduction of dietary CP from 16.5 to 12.5% reduced total nitrogen by 36% (3.23 g/kg). Ammoniacal nitrogen concentration was reduced by 43% (3.01 g/kg) and slurry pH was reduced by about 1 unit. The following regression equation was calculated to quantify the effect of dietary CP (%) on ammoniacal nitrogen concentration (g/kg):

$$[Ammoniacal nitrogen] = -5.44 (\pm 1.81) + 0.75 (\pm 0.13) \times CP; \qquad (R^2 = 0.96) \tag{1}$$

The effects of dietary CP on ammonia emission from slurry over 7 days are illustrated in Figure 1. The overall mean of ammonia emission during the 7-day measurement period was 0.57 g/d. Mean ammonia emissions for the diets with 16.5, 14.5 and 12.5% CP were 0.72, 0.56 and 0.43 g/d, respectively (P < 0.001). Total ammonia emission over the 7-day measuring period was strongly affected by dietary CP (P < 0.001). For each percent reduction in dietary CP, total ammonia emission was reduced by about 10%. The effect of dietary CP (%) on ammonia emission from slurry (g/d) was quantified with the following regression equation:

Ammonia emission = 
$$-0.47 (\pm 0.17) + 0.072 (\pm 0.01) \times CP;$$
 ( $R^2 = 0.96$ ) (2)

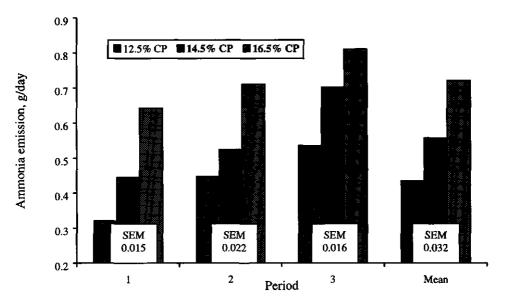


Figure 1. Ammonia emission from slurry of pigs fed different diets in the balance experiment

#### Barn experiment

The average daily weight gains, feed intake, feed conversion ratio, and carcass yield characteristics did not differ between diets (P > 0.05). Due to the effect of water dilution, slurry DM concentration was lower than that observed in the balance experiment (Table 4). Ash concentration was similar to that in the balance experiment. However, total nitrogen and ammoniacal nitrogen concentrations and slurry pH were lower than those found in the balance experiment. Total nitrogen and ammoniacal nitrogen concentrations decreased when dietary CP decreased. On average, slurry pH decreased by 0.66 unit when dietary CP level decreased from 16.5% to 12.5%.

The area of the solid floor fouled with urine was very small and differences were negligible. Ammonia emission also followed the same pattern as in the balance experiment. The overall mean ammonia emission was 7.06 g/d per pig. Ammonia emission was the highest with the 16.5% CP diet and the lowest with the 12.5% CP diet. Mean ammonia emissions over three periods for 16.5, 14.5 and 12.5% CP diets were 9.44, 6.94 and 4.79 g/d per pig, respectively. Ammonia emission was reduced by 50% when dietary CP levels decreased from 16.5 to 12.5%.

Figure 2 shows a positive linear relationship between ammonia emissions measured in barn and balance experiments. Dietary CP had a similar effect on ammonia emission in both balance and barn experiments

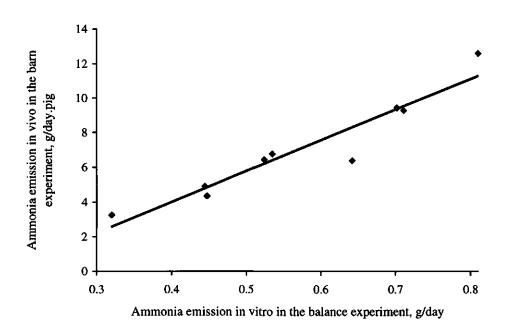


Figure 2. Fitted and observed relationships between ammonia emission in the barn (g/d.p) and balance experiments (g/d).

#### Discussion

#### Pig performance, nitrogen intake and nitrogen excretion

The results of this study support the concept that reducing dietary CP content, whilst maintaining similar levels of essential amino acids, leads to a reduction in the amount of nitrogen excretion. The supplementary amino acids were lysine, methionine, threonine and tryptophan, because they are commonly limiting in barley-based diets (Fuller et al. 1979; Valaja and Alaviuhkola, 1993). Similar results have been also obtained by some other workers (Latimier and Dourmad, 1993 and Valaja and Alaviuhkola, 1993). Tuitoek et al. (1997) reduced the dietary CP from 16.6 to 13% without any negative effect on growth rate, feed intake, feed efficiency, or carcass characteristics. In addition to this, Gatel and Grosjean (1992) observed a decrease in urinary nitrogen but not in faecal nitrogen excretion when lowering dietary CP from 16.9 to 15.6% and 14.6 to 13.5% for growing and finishing pigs. In our experiment reducing soybean meal level lowered dietary CP. Dietary nitrogen digestibility primarily depends on raw ingredients used in formulation (Gatel and Grosjean, 1992). Because soybean meal is the most digestible fraction in our diet (CVB, 1994), its reduction resulted in lowering nitrogen digestibility of the diet. Our performance results indicate that the utilisation of free and protein-bound

amino acids were not much different. With a similar amounts of daily nitrogen retained and nitrogen excreted in faeces, most of the increased daily nitrogen intake in pigs fed high CP diets was excreted in the urine. This implies that reduction of total nitrogen excretion was mainly caused by the reduction in urinary nitrogen excretion.

#### Composition of the slurry

Slurry amount was very similar between diets. This was probably caused by a fixed amount of water supplied to the pigs. In normal situations, ad libitum water supply may result in different water intake and thereby slurry amount will be different. Kay and Lee (1997) found an 11% decrease in slurry amount for each percent reduction of dietary CP. The present study demonstrates that dietary CP affected slurry composition. Lowering dietary CP reduced ammoniacal nitrogen content and consequently, decreased total nitrogen content. This is in agreement with the findings of Kay and Lee (1997) and Sutton et al. (1997). According to Canh et al. (1997a), ammonia in slurry is mainly produced from urinary urea. Urea nitrogen represents more than 95% of total nitrogen in pig urine. Therefore, in this study, the decrease in urinary nitrogen excretion was the main explanation for the low content of ammonia in the slurry from pigs fed the low CP diet. Within diets, total nitrogen and ammoniacal concentrations of the slurry in the barn experiment were somewhat lower than those observed in the balance experiment. This is probably because in the barn experiment, slurry was sampled at the end of the period in which ammonia emission was measured, and it is likely that a significant amount of ammonia was lost by volatilization during that period. The difference in total nitrogen and ammoniacal contents between the two experiments was higher for the diet with high CP content. This means that during storage, the slurry of pigs fed high CP diet lost more nitrogen than that from pigs fed low CP diet.

Dietary CP strongly influenced slurry pH. Sommer and Husted (1995) reported that ammonium concentration of slurry is one of the main factors influencing slurry pH. Therefore, it is suggested that the lowered slurry pH in this study was mainly caused by the lower ammonium content of slurry from pigs fed a lower CP diet. However, the pH was in general higher than those observed by Canh et al. (1997a, b), Sutton et al. (1997). A possible explanation for this high pH could be the low level of dietary non-starch polysaccharides (Canh et al., 1997a). In addition, the use of virginiamycin in the diet might prevent a saccharolytic and favouring a proteolytic fermentation in the slurry.

#### Ammonia emission

Ammonia emission into the air is influenced by factors such as ammonium concentration, pH and temperature (Muck and Steenhuis, 1981; Freney et al, 1983; Stevens et al., 1989; Aarnink, 1997). In the slurry, ammonia (NH<sub>3</sub>) is in equilibrium with ammonium (NH<sub>4</sub>). This balance turns to ammonia at higher pH value and hence favours ammonia emission. In this study, at a fixed temperature ammonium content of the slurry was the main factor influencing pH and ammonia emission. Lowering dietary CP lowered ammonia concentration and slurry pH, consequently reduced ammonia emission. Similar results were

obtained by Key and Lee (1997), who found a 9.8% reduction of ammonia emission from slurry for each percentage decrease of dietary CP. Reductions in ammonia emission are also in line with predicted data from Latimier and Dourmad (1993), Oldenburg and heinrichs (1996) and Cole et al. (1996).

The current study is one of few in which ammonia emissions were measured directly both in vitro and in a practical pig house. It furthermore indicates that reduction of ammonia emission can be obtained in both situations. This implicates that the results of ammonia emission measured in vitro gave a very good indication of the results that can be obtained in a real situation. Pain et al. (1997) reported that ammonia emission from pig buildings represented more than 50% of ammonia emission from pig sector. Reducing ammonia emission from pig houses by 50%, as achieved in this study, would considerably contribute to limiting ammonia emission from pig production system.

#### Conclusion

Reducing the dietary protein and supplementing the diet with essential amino acids whilst maintaining the normal level of pig performance, significantly reduced the total and urinary nitrogen excretion and the ammonium concentration of slurry of growing-finishing pigs. This consequently reduced the ammonia emission from the slurry. This may be an economical way to reduce ammonia emission from pig production systems.

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Chapter 9

# **GENERAL DISCUSSION**

## **General discussion**

#### Introduction

Ammonia volatilisation from slurry decreases the slurry's fertiliser value. Furthermore, the deposition of the ammonia emitted into the atmosphere may cause undesirable changes in aquatic and terrestrial ecosystems (Apsimon and Kruse-plass, 1990). In pig husbandry, volatilisation of ammonia is also a main factor affecting air quality inside pig buildings (Morrison et al., 1993, Aarnink, 1997). Therefore, many attempts have been made to reduce the ammonia emission into the environment to an acceptable level. For sustainable animal production, it is important to evaluate the feasibility of reducing ammonia emission in all steps of the production chain. The manipulation of the animals' feeding may be considered as the first intervention in this chain: "reduction of ammonia emission from animal production systems starts from the animals by means of nutrition".

The focus of the researches described in this thesis was to reduce the emission of ammonia from the slurry of growing-finishing pigs. The main objective was to determine the effect of different dietary factors on excreta composition, slurry characteristics, and ammonia emission. The underlying principle was to reduce ammonia emission but maintain normal animal performance. This was to result in effective and economical solutions to enable pig farmers to reduce the ammonia emission.

Ammonia is a conversion product of urea in urine. Urea is converted into ammonia and carbon dioxide by the enzyme urease present in faeces. The ammonium concentration and pH of slurry are the most important factors influencing the ammonia emission from pig houses (Muck and Steenhuis, 1981; Freney et al., 1983). The experiments described in this thesis tested the effectiveness of three main strategies to reduce ammonia emission from pig slurry by altering dietary composition.

The first strategy was to shift nitrogen excretion from urine to faeces. In the studies described in Chapters 2, 3, 5 and 6, the diets were composed on the basis of a similar level of protein but differed in non-starch polysaccharide (NSP) content. It was hypothesised that urinary excretion of urea would be reduced when more NSP was included in the diet.

In the second strategy, the ammonia emission was reduced by lowering slurry pH by changing the pH of urine and/or faeces. Urinary acidity is the result of renal regulation of the acid-base status in the animal (Patience et al., 1987). The effects of dietary electrolyte balance (dEB) on the pH of urine and slurry are presented in Chapters 2 and 3. In these Chapters the effect of dEB in combination with the effect of dietary NSP on the pH of urine and faeces, and slurry is described. The effects of dEB as an independent factor and acidifying Ca-salts on the pH of urine and slurry are reported in Chapter 7. It was expected that the pH of urine and slurry would reduce in response to lowering dEB level. From Chapters 2 to 6, the effects of dietary NSP on the pH of faeces and slurry are evaluated. The main concept of these

investigations was that the pH of faces and slurry could be reduced by increasing the bacterial formation of volatile fatty acid (VFA) in the hind gut of pigs and in the slurry during storage. To do this fermentable carbohydrates were included in the diet.

The third strategy aimed to reduce the ammonia emission by decreasing the urinary nitrogen excretion by decreasing the crude protein content of the diet. The effect of different dietary crude protein levels on nitrogen excretion and ammonia emission is described in chapter 8. It was expected that lowering dietary level of crude protein in combination with supplementing essential amino acids would reduce the total and urinary excretion of nitrogen. Reduction of urinary nitrogen was mainly caused by the reduction of urinary urea excretion.

Another aspect considered was whether the reduction of ammonia emission measured in vitro could be achieved in the dynamic situation in real pig houses. Chapter 8 described how the effect of diet on ammonia emission measured in the in vitro system was validated in real pig houses. It was expected that data on ammonia emission from in vitro measurements gave a good estimation of those observed in a practical situation.

In this chapter the feasibility of reducing ammonia emission by nutritional means is discussed. The economics, prospects and the implications of the alterations in pig feeding strategy on animal welfare and health and the working conditions of the stockman are indicated. Finally, the main conclusions from the studies described in the thesis are given.

#### Reducing ammonia emission by shifting nitrogen excretion from urine to faeces

The pigs use ingested protein for body synthesis. In addition to the requirement for new tissue formation, some protein is required for maintenance. Part of the dietary protein is indigestible and excreted in faeces. However, a much higher proportion of the pig nitrogen excretion appears in the urine. According to Jongbloed and Lenis (1992), the average slaughter pig excretes about 50% of the intake nitrogen via urine and about 20% via faeces. The average ratio of urinary nitrogen and faecal nitrogen (NER) found in our research (Chapter 2) was about 2.64. This ratio differed between diets, ranging from 1.21 to 3.83, although nitrogen intake and total nitrogen excretions were similar for all diets. Dietary NSP was the main factor causing differences in nitrogen excretion pattern. When more NSP was included in the diet, there was a shift of nitrogen excretion from urine to faeces. This evidence was firstly reported by Morgan and Whittemore (1988) and recently by Mroz et al. (1993), Schulze et al. (1993) and Kirchgessner et al. (1994). Our later studies (Chapters 4 and 6) have again confirmed this evidence.

The nitrogen excreted in faeces is predominately incorporated in bacterial protein, which is very resistant to further degradation. Nitrogen in the urine is mainly excreted in the form of urea. Urinary urea is easily hydrolysed to ammonia and carbon dioxide, especially when the urine comes into contact with faeces on the solid floor or in the slurry pit in pig houses. It is obvious that reduction of urinary nitrogen excretion reduces a volatilisable form of nitrogen: ammonia. This results in a reduction of ammonia volatilisation from slurry in pig houses.

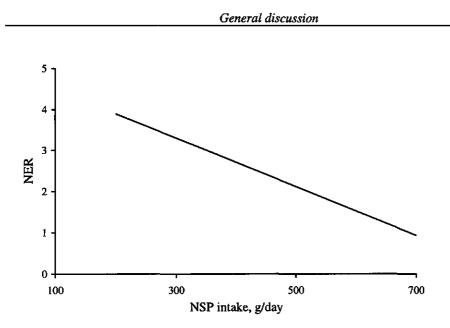


Figure 9.1. Relationship between intake of dietary nonstarch polysaccharides and nitrogen excretion ratio (Chapter 2)

$$\begin{split} NER &= 5.085(\pm 0.973) - 0.00593(\pm 0.00222) \times NSP(g/day), & [R^2 = 0.93] \\ UrinaryN(g/day) &= 35.08(\pm 4.32) - 0.0278(\pm 0.0029) \times NSP(g/day), & [R^2 = 0.93] \\ FaecalN(g/day) &= 4.10(\pm 1.38) + 0.0148(\pm 0.003) \times NSP(g/day), & [R^2 = 0.96] \end{split}$$

Increasing NSP in the diet was found to reduce urinary urea concentration and slurry ammonium concentration (Chapters 2 and 5), and therefore reduces the ammonia emission from slurry.

In principle, pigs can not digest fibre. The colonic microflora degrades a major part of fibrous components of the diet and uses their products as the main energy sources for microbial activity (Malmlöf and Håkanssom, 1985). The urea secreted from the blood into the large intestine serves as a nitrogen source for microbial growth (Low, 1985; Sauer et al., 1991). This microbial protein is finally excreted in the faeces. This effect of dietary fibre on the ability of micro-organisms to retain nitrogen within the gut also demonstrates to depress urinary nitrogen output.

The results from our studies showed that nitrogen excretion ratio could best be described by linear regressions (Figure 9.1). For each 100 g increase in dietary NSP intake, The NER ratio reduced by 0.6 unit, urinary nitrogen reduced by 9% (2.78 g/day) and total urinary urea reduced by 10% (2.60 g N/day). Both the total amount and urea concentration of urine reduced remarkably when dietary NSP intake was increased. Faecal nitrogen is mainly incorporated in biomass of the large intestine. The increase in biomass is the result of nitrogen excretion shifting from urine to facces when more NSP are included in the diet.

Response variable	Explanatory variable (β)	Constant ± SE	$\beta^a \pm SE$	P <sup>b</sup>	R <sup>2c</sup>
NER <sup>d</sup> (unit)	CP (%)	-2.34 ± 1.11	$0.35 \pm 0.08$	< 0.001	0.99
NH4 <sup>+</sup> -N (g/kg)	CP (%)	-5.44 ± 1.81	$0.75 \pm 0.13$	< 0.001	0.97
Emission (mmol)	CP (%)	-27.6 ± 9.80	4.22 ± 0.67	< 0.001	0.96

Table 9.1. Relationships between dietary CP, slurry ammonium concentration, urinary N
and faecal N excretion ratio and ammonia emission from slurry (Chapter 8)

<sup>a</sup>Regression coefficient.

<sup>b</sup>Significance level.

<sup>c</sup>Correlation coefficient.

<sup>d</sup>Urinary N and faecal N excretion ratio.

This change in nitrogen excretion pattern not only reduces ammonia emission from slurry but also benefits animal health by reducing the formation of urea in the liver and the excretion of urea via kidney.

The amount and composition of NSP (cellulose, hemicellulose and lignin) are important factors influencing microbial fermentibility. In our studies, we found that the amount and sources of NSP both affected nitrogen excretion pattern in pigs. The largest reduction of ammonia emission was obtained when soybean hulls (Chapter 4), sugar beet pulp (Chapters 2 and 3) or beer by-products (Chapter 5) were included in the diet. Reduction of ammonia emission is positively related to the level of cellulose and hemicellulose. High lignin content in the diet depresses the reduction of ammonia emission.

There are a wide variety of feedstuffs available for pig diets, differing not only in nitrogen content but also in non-nitrogen fractions. Dietary manipulation by increasing the amount of high-NSP ingredients in the diet of pigs to reduce the ammonia emission from pig slurry did not reduce the daily weight gain of pigs. Most of the high-NSP feedstuffs are by-products from agricultural, food and beverage processing industries. In present studies, the NSP sources used were sugar beet pulp, soybean hulls, coconut expeller, rice bran and beer by-product, which are commonly used in commercial pig feeding. It is clear that alteration to these low-cost by-products instead of grains such as barley, wheat, maize starch or tapioca in the diet benefits not only in terms of environmental aspects. There certainly is less competition between animal and human consumption in term of nutrient supply.

#### Reduction of ammonia emission by lowering dietary nitrogen supply

In growing pigs, the amino acid composition of an ideal protein represents the balance in which amino acids are required for maintenance and body protein accretion (Fuller et al., 1989; Lenis et al., 1993). The pigs need protein in term of amino acids rather than total supply of protein. An ideal-protein diet provides a pattern of amino acids for the pigs in a proportion that is biologically required by the animal. The absorbed amino acids that are not used for tissue protein synthesis are deaminated to volatile fatty acids and ammonia. This ammonia is finally excreted as urea in urine. Therefore, oversupply of protein or amino acid-unbalanced-protein supply in the diet leads to an increase of the excretion of nitrogen in urine. For economically viable and environmentally acceptable pig production, it is essential to provide a well-balanced diet. This diet should support not only environmental aspects but first of all, ensure an adequate and efficient growth of the animal. Reduction of dietary nitrogen can reduce urinary nitrogen excretion. However, the reduction of dietary nitrogen content must be undertaken carefully. Essential amino acids must be supplied in accordance with the pig 's requirement. Lenis (1989) showed that lowering the crude protein level by one percentage unit in the diet for growing-finishing pigs decreased nitrogen excretion by 8.5%. At the low protein level, synthetic lysine and methionine were included in the diet. In our study (Chapter 8), a further substantial reduction in nitrogen excretion was obtained by reducing protein level by 4%, from 16.5% to 12.5%. For one percentage unit reduction of dietary protein, nitrogen excretion was reduced by about 9%. In addition to lysine and methionine, synthetic threonine and tryptophan were also added to the low crude protein diets. In this study, lowering dietary protein mainly reduced nitrogen excretion in urine but not in the faeces.

So far, most of research has focused on reducing nitrogen excretion by lowering dietary crude protein (Lenis, 1989; Lenis et al., 1993; Fremaut and De Schrijver; 1990; Tuitoek et al., 1997). In our study the effect of dietary protein on ammonia emission was also quantified. Because most of the urinary nitrogen is in the form of urea, the reduction of urinary urea leads to a reduction of ammonium content in the slurry of pigs fed a low crude protein-contained diet. The dry matter content of the slurry was not affected by reducing dietary crude protein. It was the reduced ammonium content of the slurry that was largely responsible for the reduced ammonia emission from slurry. Reducing dietary protein by one percentage unit reduced the NER ratio by about 0.35 unit, the ammonium concentration of slurry by about 11.4% (0.75 g/kg slurry) and ammonia emission reduced by about 10% measured in vitro, and about 12.5%, measured in a pig house. The relationships between dietary CP, slurry ammonium concentration, NER and ammonia emission from slurry are best quantified in the linear regressions in Table 9.1. In this study, water supply was restricted. Kay and Lee (1997) reported a reduction of slurry production by 11% for each percentage unit reduction of dietary crude protein. This was caused by the low water intake of animals' fed the diets with low crude protein level. In real pig houses, with the same surface area of slurry in the pit, ad libitum water supply may cause differences in slurry production and ammonium concentration. These differences may also influence ammonia emission.

In the present study, reducing dietary protein from 16.5% to 12.5% did not affect animal weight gain, feed conversion efficiency and carcass yield characteristics. Thus the efficiency of using synthetic amino acids for body protein accretion at 12.5% crude protein level was not different from using intact protein in the 14.5% or 16.5% crude protein content diet. On similar body weight pigs, Tuitoek et al. (1997) found that feed efficiency was reduced when pigs were fed a diet containing 11% crude protein compared with pigs fed diets containing 14.2 and 12.8% crude protein.

VFA	Low carbohydrate intake (mmol/day)	High carbohydrate intake (mmol/day)
Acetate	97.5	129.2
Propionate	33.3	40.9
Butyrate	11.6	18.2
Total VFA	142.4	188.3

#### Table 9.2. Daily excretion of faecal volatile fatty acids (VFA) in pigs fed low and high levels of carbohydrates (Imoto and Namioca, 1978)

The 12.5% crude protein level could be an optimal environmental and economical option for finishing pigs, because a large reduction of ammonia emission can be expected whilst maintaining production levels.

The second approach to reduce nitrogen excretion is phase feeding. In our study (Chapter 8), we found that when the pigs reached about 80 kg BW (finishing phase), daily gain declined and the increased nitrogen intake was mainly excreted in urine. The results demonstrate the potential for further reducing ammonia emission by using phase feeding.

#### Reducing ammonia emission by lowering the pH of slurry

The pH of slurry strongly affects the ammonia volatilisation (Freney et al., 1983; Stevens et al., 1989; Sommer and Husted, 1995). Because the effect of the pH is very strong, a minor change in pH can have a large effect on ammonia emission. Some researchers have reduced the pH of slurry by acidification. Stevens et al. (1989) reported that lowering slurry pH by 1 unit, from 7 to 6, with sulphuric acid reduced the ammonia emission from pig slurry by 82%. A similar result was also found by Hoeksma et al. (1993). However, lowering the pH of slurry by acidification does not seem to be feasible, because of the high costs and technical applicability, and furthermore, the use of inorganic acids may give other environmental problems. Therefore, it is necessary to look at other ways of reducing the pH of slurry. Dietary manipulation to change the slurry characteristics may be a good option.

Theoretically, the pH of the slurry is determined by the concentration of hydrogen cations in the slurry. Hydrogen cations, in turn, largely depend on the total anion-cation balance of the slurry. According to Sommer and Husted (1995), the pH of slurry is mostly determined by the concentrations of NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub>, CO<sub>2</sub>/HCO<sub>3</sub><sup>-/</sup>CO<sub>3</sub><sup>-/2</sup> and VFA. Paul and Beauchamp (1989) found that the pH of slurry was highly correlated (R<sup>2</sup> = 0.95) in a linear relation ( $\beta$  = 2.02) with (VFA)/(NH<sub>4</sub><sup>+</sup> + NH<sub>3</sub>) of the slurry. Increasing the VFA concentration lowers the slurry pH and increasing the total ammonium concentration

raises the slurry pH. In our studies, reductions of slurry pH were obtained through changing the composition and hence the pH of urine and/or faeces.

The pH of faeces and slurry was reduced drastically when more NSP was included in the diet of pigs. A high level of dietary NSP enhances microbial activities in the hind gut of pigs, increasing VFA formation in faeces and in the slurry during storage. It is obvious that, increasing the intake of NSP linearly increases the VFA concentration in faeces and in the slurry. Acetic, propionic and butyric acids are predominant in the VFA pool of the slurry. The other acids are usually present in small amounts (about 5-10%). Imoto and Namioca (1978) reported that the excretion of faecal VFA increases in response to increasing dietary fermentable carbohydrates (Table 9.2). In addition to these findings, the absorption rate of acetic acid in the pigs reduces at higher levels of dietary NSP. This means that increased NSP in the diet results in an increased excretion of acetic acid, the most active fatty acid, in faeces. During storage of slurry, the decomposition of plant fibre residues to VFA is the most important process (Spoelstra, 1979). There is still lack of information on the effect of dietary NSP on the pH of and ammonia emission from the slurry. In our studies, we found a high correlation between dietary NSP and pH and ammonia emission. The deposition of VFA in slurry originating from microbial fermentation in the hind gut of animals and in the slurry during storage reduces the pH of the slurry. As a consequence, ammonia emission is reduced. The ammonia emission from slurry is significantly correlated ( $R^2 = 0.75$ ) with the intake of NSP.

The level of NSP in the diet can be increased from 30 (from sugar beet pulp; chapters 2 and 3) to about 50 % of the feed dry matter (from soybean hulls or sugar beet pulp; chapter 4). Dietary NSP influences the ammonia emission in two ways. On one hand, it increases VFA formation and reduces the pH of slurry. On the other hand, the inclusion of NSP in the diet shifts N excretion from urine to faeces. The pH of slurry lowered by about 0.12 unit and ammonia emission reduced by about 5.4% for each 100 g increase in the intake of dietary NSP (Chapter 4). The reduction of urea excretion caused by dietary NSP significantly contributes to a reduction of ammonia emission. For each 100 g increase in the intake of NSP the NER ratio reduces by about 0.6 unit (Chapter 2). Ammonia emission is very highly correlated with the NER. If this reduction in urinary N excretion is taken into the account, the reduction of ammonia emission will be obtained at higher levels.

The most common NSP sources that can be used are sugar beet pulp, coconut expeller, soybean hulls or beer by-product. At restricted feeding as in our studies, it is possible to include 50% of those by-products in the diet of growing-finishing pigs. Ammonia emission can be reduced by about 40%, while maintaining a normal growth of the pigs.

The pH of slurry can also reduce in response to the pH of urine. Elzing and Aarnink (1996) estimated the effect of the urinary pH on the ammonia emission in a model situation of a pig house. They found ammonia emission reduced by about 12% when urinary pH lowered from 7 to 6. However, so far no research has been done on quantifying the effect of dietary factors on the pH of urine and slurry related to the ammonia emission. In our study (Chapter 7), we hypothesised that by reducing dietary electrolyte balance and/or adding acidifying Ca salts to the diets for pig, urinary pH would be decreased,

dEB (meq/kg)						
Variable	320	100	CO <sub>3</sub>	SO4	Cl <sub>2</sub>	Benzoate
Urinary pH	6.02 <sup>A</sup>	5.56 <sup>B</sup>	7.05ª	5.44 <sup>b</sup>	5.39 <sup>6</sup>	5.25 <sup>c</sup>
Slurry pH	7.45 <sup>A</sup>	7.31 <sup>B</sup>	8.16 <sup>a</sup>	7.34 <sup>b</sup>	7.30 <sup>b</sup>	6.63 <sup>c</sup>
NH <sub>3</sub> emission (%)	100	89	100	70	67	46

Table 9.3. Dietary electrolyte balance and acidifying Ca salt influencing	
urinary pH, slurry pH and ammonia emission from slurry	

A.B; a,b,c Means within a row lacking a common superscript letter differ (P < 0.05).

and thereby, slurry pH and ammonia volatilisation from the slurry would also fall. Our high dietary electrolyte balance (dEB) diet was chosen as a practical control. Lowering dEB from 320 to 100 meq/kg DM of feed reduced urinary pH by about 0.46 unit, slurry pH by about 0.14 unit and ammonia emission by 11%.

Adding Ca-benzoate,  $CaCl_2$  or  $CaSO_4$  to the diet instead of  $CaCO_3$ , a normal Ca salt used in most commercial diets for growing pigs (Mroz et al., 1996), lowered the urinary pH by 1.80, 1.66 and 1.61 units, respectively. Slurry pH lowered by 1.53, 0.86 and 0.82 units and ammonia emission by 54, 33 and 30%, respectively (Table 9.3). Adding Ca-benzoate in the diet gave the largest reduction of pH and ammonia emission. Dietary acidifiers can increase renal acid excretion and reduce the pH of the urine. The anion-cation balance (dEB) is an important factor affecting the acid-base status in the animal. Urine acidity is the result of renal regulation of the acid-base balance in the animal.

The pH of slurry can be reduced by simultaneously influencing both the pH of urine and of faeces. Dietary manipulation of dEB and NSP levels (Chapters 2 and 3) shows clear effects on the pH of slurry and ammonia emission. Reducing dEB from 250 to 120 meq/kg in feed and increasing dietary NSP from 13 to 31% lowered the pH of slurry by 0.8-0.9 unit and ammonia emission by 52-53%. This reduction was obtained by adjusting dietary ingredients that are commonly used in commercial pig feeding.

#### Improving fertiliser quality through reduction of nitrogen losses

The uptake of organic nitrogen by plants is a very important step in the global nitrogen cycle. For sustainable agricultural production, it is necessary to integrate livestock and crop production into a unified system. In many countries, especially developing countries, livestock residues are still very important in the agricultural production chain. Manure is a valuable nutrient source for vegetation and for soil bacterial growth. Nitrogen leaching and run off from the soil and the volatilisation of ammonia from manure spread on land reduce its fertiliser value. Depending on the method of application, ammonia volatilisation from pig slurry accounts for about 18% of the total nitrogen ingested and about

26% of total nitrogen excreted by the growing-finishing pigs (Aarnink, 1997).

Three main groups of factors affect ammonia volatilisation from the spreading of livestock manure (Svenson 1994): 1) meteorological, 2) soil/application technique and 3) the manure. The characteristics of manure such as dry mater and ammonium concentrations, and the pH, affect the rate of releasing ammonia is released into the air (Svenson, 1994). Changes in excreta composition resulting from changes in dietary composition are expected to impact on the nitrogen dynamics of manuring. Buijsman et al. (1986) reported that about 90% of nitrogen losses from manure during application to the soil originate from urine. The present studies show that feeding manipulation can modify slurry characteristics, resulting in a reduced ammonia emission and giving high quality pig manure. Shifting nitrogen from urine to faeces and lowering the pH of slurry can be considered as the first step in improving manure quality. The results from our studies demonstrate that the modification of slurry characteristics reduces the ammonia emission from slurry during storage. This improves the quality of pig manure before application to arable lands or pasture. Those changes of slurry characteristics to sustain nitrogen in manure might decrease nitrogen losses through emission of ammonia after spreading manure on the soil as well. However, it is better to combine these feeding measures with application methods such as injection or ploughing in order to reduce ammonia emission.

Further research is needed to reveal how this manure can best be used by crops, while minimising the environmental impact on the ecosystem. Improving the slurry by maintaining the nitrogen nutrient in the manure would contribute to the soil nutrient balance and allow farmers to reduce application of artificial fertiliser. The soil biomass could also be improved. Overall, this new approach of feeding could be applied in a modern mixed farm operation characterised by intensive co-operation between various agricultural production sectors, resulting in low input of artificial fertiliser and reduced environmental impacts and thus contribute to sustainability of agriculture.

# Feasibility of altering nutrition as a means to reduce environmental pollution from pig farming

The primary causes of the high ammonia emissions from pig production systems are the high number and density of pigs and inappropriate feeding. Nutrition can contribute substantially to reducing ammonia emission in different ways. Excessively protein-rich feeding generally increases nitrogen excretion and therefore, increases ammonia emission. A better agreement between supply and the requirement of pigs is a good way to obtain a balance between production and environment. Results from our studies demonstrate that the best balance can only be obtained by a balanced combination of nitrogen and non-nitrogen components in pig diets. This can also improve the context of bacterial biomass, especially the C/N ratio in the hind gut of pigs as well as in the slurry during storage.

Although in our studies we did not evaluate the costs of alterations of dietary composition, reduction of ammonia emission through feeding seems to be achievable at relatively low costs. There

are a number of ways to reduce ammonia emission. Compared with other techniques such as air treatment and manure treatment systems, dietary manipulation seems to be a simple way. The addition of synthetic amino acids or some Ca salts in the diet might increase feed costs. Ca-benzoate proved to be very effective in reducing the pH and ammonia emission. However, this product is not yet on the list of permitted additives for pig nutrition. More research is needed on the intermediary metabolism and safety of Ca-benzoate. In our research, the effects of dietary factors on animal performance and ammonia emission were mostly assessed in a short time period with restricted feeding. Further research is needed to determine the effects on animal health and production in the long term. Also, the effects of dietary factors on ammonia emission might differ during long slurry storage. Generally, in our study, diets were altered by adjusting component ingredients that are widely available for commercial pig feeding. Most of them are cheap by-products from agricultural and food and beverage industries. Using those byproducts instead of grains will certainly reduce feed costs. Lowering ammonia emission by altering dietary compositions does not need sophisticated techniques. Therefore, this can reduce cost per unit of production.

Depending on the types of diets, in our studies the ammonia emission from slurry can normally be reduced by about 50%. In Chapter 8, the effect of the diets on ammonia emission was tested in both in vitro scale and in a practical pig house. A similar reduction of ammonia emission was obtained in both situations. The in vitro system simulates the storage of slurry in the pit. However, ammonia is also emitted from fouled solid and slatted floors. Aarnink and Elzing (1998) estimated floor emission varied from 30-42%, depending on the type of slatted floor. It might be expected that lowering the urea concentration and the pH of urine will reduce the ammonia emission from the floor as well. The influence of a lower pH faeces pH on the floor emission might be small, because urine and faeces are not mixed on the floor. It is therefore recommended to validate the most promising dietary alterations on ammonia emission in practical pig houses. Schrama et al. (1996) reported that increasing the level of sugar beet pulp silage in the diet of growing-finishing pigs to 15% of DM, as was done in our study (Chapter 6) significantly decreased pigs' activity. The decreased activity might result in a reduction of dust concentration in pig houses. Lowering the ammonia and dust concentrations in the room air would improve the indoor conditions, which will benefit pig health and the working conditions of pig farmers. So far very little research conducted on the relationship between dietary composition and odour emission. Because many of the odorous compounds are nitrogenous, the incorporation of nitrogen in bacterial biomass may influence the formation of these compounds. Reduction of nitrogen excretion and changing the slurry pH may also affect the formation and emission of these odorous compounds. Further research in this area is needed to evaluate the effects of dietary composition on odour emission from pig excreta.

In our concept, an ideal diet, which can assure the optimal performance of the pigs and obtain the required reduction of environmental impact of pig' excreta, can be formulated to contain from 12.5% to 16.5% of crude protein. Dietary level of crude protein can be reduced by supplementing with essential limiting amino acids and/or phase feeding. The levels of NSP in the diet can be altered from 20 to 30% depending on pig growth period and source of NSP. The level of dEB can be reduced to 100 meq/kg. Calcium can be supplied in the form of CaSO<sub>4</sub> or CaCl<sub>2</sub> instead of CaCO<sub>3</sub>. Austic et al. (1982) found no

General discussion

differences in average daily gain and feed conversion ratio in pigs fed diets with dEB ranging from -100 to 500 meq/kg. Den Hartog et al. (1989) noted that the level of dEB influences the growth of pigs only when amino acids are limiting. From our studies, it is clear that a reduction of 50 to 70% of ammonia emission can achieved, while maintaining normal performance of the pigs.

## **General conclusions**

- Feeding influences the rate ammonia is released from slurry in pig houses. The difference in ammonia emission depends not only on the nitrogen components in the diet but also on the nonnitrogen components such as nonstarch polysaccharides and minerals. Therefore, it is necessary to consider ammonia emission reduction from the first step: *start from the animal and by means of nutrition*. Altering the diet changes the slurry characteristics, resulting in lower ammonia emissions.
- Lowering dietary crude protein level combined with supplementation of essential amino acids to the pigs reduces urinary nitrogen excretion, ammonium content of slurry and the ammonia emission from the slurry. The acceptable crude protein level of the diet can be 12.5% for finishing pigs. This can combine a normal performance of the pigs with a large reduction of ammonia emission from the pig house.
- Increasing the level of nonstarch polysaccharides in the diet has double effect on reducing the ammonia emission. First, by shifting nitrogen excretion from urine to faeces, and secondly, by reducing slurry pH via an increase in volatile fatty acid formation in the pig and in the slurry.
- Lowering the dietary electrolyte balance (Na + K Cl) and/or adding acidifying Ca salts to the diet reduces the pH of urine and slurry and the ammonia emission from slurry. The electrolyte balance can be decreased to 100 meq/kg DM without adversely affecting pig performance. Calcium supplied in the form of CaSO<sub>4</sub> or CaCl<sub>2</sub> instead of CaCO<sub>3</sub> gives a strong reduction of ammonia emission. Adding Ca-benzoate to the diet instead of CaCO<sub>3</sub> gives the largest reduction of ammonia emission. However, further research should be done on the safety use of Ca-benzoate for the pigs.
- Reducing ammonia emission through dietary manipulation can be a simple and economical way for farmers to reduce the environmental impact of pig production. Furthermore, the indoor air quality in the pig houses can be improved. This will improve animal welfare and the working conditions of the pig farmers.
- An increase in stable forms of nitrogen in pig slurry and a lowering of the pH of the slurry can improve the fertiliser quality of pig manure. This may contribute to sustainable farming systems with limited impact on the environment.

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# SUMMARY

# SAMENVATTING

## Summary

Ammonia volatilisation from pig production systems is undesirable. It decreases the fertiliser value of slurry. Furthermore, deposition of the emitted ammonia from the atmosphere may cause undesirable changes in aquatic and terrestrial ecosystems. There are a number of factors influencing ammonia volatilisation. Of these, ammonium concentration and pH of slurry are very important. The objective of this study was to evaluate the feasibility of influencing these slurry factors by nutritional means, resulting in a lower ammonia emission from the slurry. This goal was obtained by three main ways:

1. Shifting nitrogen excretion from the easy volatile form in the urine to the less accessible form in the faeces.

2. Lowering the pH of urine and/or faeces to decrease the slurry pH.

3. Lowering the total nitrogen excretion by the pig by lowering the nitrogen input.

The experiments were performed with growing-finishing pigs. The main condition of the project was that these solutions should not have any adverse effects on animal health and performance or produce other negative effects on the environment.

In Chapter 1 the objectives of the thesis are described on the basis of the problem definition. Chapters 2 and 3 describe preliminary experiments in which different diets were formulated, which mainly differed in levels of nonstarch polysaccharides (NSP) and electrolyte balance (dEB = Na + K - KCl). Their effects on the nitrogen excretion pattern, the pH and ammonium content of the slurry and on the resulting ammonia emission were determined. Generally, by-products from human food-industry are relatively cheap components to be used in pig diets. These by-products often contain a high level of NSP. Three experiments investigated the effects of these by-products in the diet on the ammonia emission. In the first experiment, described in Chapter 4, the effects of source and level of dietary NSP on the pH of faeces and slurry and on ammonia emission were quantified. In this study maize starch in the diet was replaced at three levels by coconut expeller, soybean hulls and dried sugar beet pulp. The second experiment was performed in Vietnam with local by-products: coconut expeller, rice-bran and beer byproduct (Chapter 5). The third experiment was performed with sugar beet pulp silage, one of the main by-products from food industry in the Netherlands (Chapter 6). In the research described in Chapters 4 to 6 the main objective was to reduce ammonia emission by influencing the pH of facces. It was hypothesised that increasing the level of NSP in the diet enhances the formation of volatile fatty acids in the hind gut of pigs and in the slurry during storage, resulting in a lower ammonia emission from the slurry.

The pH of urine might be influenced by manipulating dietary electrolyte balance and/or acidifying Ca-salts. The experiment described in Chapter 7 shows the effect of dEB level and acidifying Ca salts (CaSO<sub>4</sub>, CaCl<sub>2</sub> or Ca-benzoate) in the diet of growing-finishing pigs on the pH of urine and slurry and on the ammonia emission.

Lowering the ammonium concentration of the slurry can reduce ammonia emission. The first approach in our study to lower slurry ammonium was to shift nitrogen excretion from urine to faeces, as presented in Chapters 2 and 5. Various previous studies have described the effect of nitrogen intake on ammonia emission. The researches obtained very different results in the relationship between nitrogen intake and ammonia emission. In the experiment in Chapter 8 we determined this relationship under very controlled circumstances. We further investigated whether there was an interaction between the effect of nutritional factors and climate factors on ammonia emission.

Most of experiments described in this thesis were performed with pigs in balance cages. The experiment with sugar beet pulp silage (Chapter 6) was done with group housed pigs in climate chambers. The experiment with protein level of the diet (Chapter 8) was performed with pigs in balance cages as well as with pigs in a real pig house. Ammonia emission was always measured in vitro, in a laboratory set-up. Faeces and urine, collected separately from the balance cages, were mixed to slurry and sampled for the in vitro emission measurements. The slurry produced in the climate chamber and the pig house was sampled for the in vitro measurements. The results from the in vitro measurements were validated with ammonia emission measurements from the real pig house (Chapter 8).

The feasibility of reducing ammonia emission by nutritional means and its environmentally and economically implications are discussed in Chapter 9. That chapter also contains the main conclusions of the thesis.

#### Ammonia emission and nitrogen excretion pattern

The nitrogen not retained in the pig is excreted in urine or faeces. The nitrogen excreted in faeces is predominately incorporated in bacterial protein, which is highly resistant to degradation to ammonia and other components. The nitrogen excreted in urine is mainly in the form of urea, which is easily converted into ammonia and carbon dioxide. Dietary NSP can shift nitrogen excretion from urine to faeces. In our experiments diets with different levels of NSP were investigated. The results from our studies showed that when nitrogen intake was similar, diet has no effect on total nitrogen excretion. Thus, similar levels of nitrogen were retained in the pig. However, nitrogen excretion pattern differed strongly between diets. Pigs fed diets containing more NSP excreted less nitrogen via urine and more nitrogen via faeces than pigs fed diets containing less NSP. In Chapter 2 we demonstrated that for each 100 g/d increase in dietary NSP intake, the urinary nitrogen to faecal nitrogen ratio (NER) reduced by 0.6 unit, urinary nitrogen reduced by 9% (2.78 g/d) and total urea-N reduced by 10% (2.60 g/d). This change in nitrogen excretion pattern was also found for the diets containing the NSP-rich by-products. Tapioca in the control diet was replaced by the NSP-rich coconut expeller, rice bran or beer by-product. This resulted in the NSP level of the diet ranging from 6.8 to 20.4%. The beer by-product diet contained most NSP. This diet caused the largest excretion of nitrogen via faeces, and the least nitrogen excretion via urine. Urinary nitrogen excretion, mainly in the form of urea, is strongly related to the ammonium content of the slurry (Chapters 2, 5). Reduction of urinary urea resulted in a decreased slurry ammonium concentration, consequently lowering ammonia emission from the slurry.

#### Ammonia emission and slurry pH

The pH of slurry is a very important factor affecting the ammonia emission. Theoretically, it is determined by the concentration of the hydrogen cation in the solution. Hydrogen cation, in turn, depends extensively on the total anion-cation balance of the slurry. The concentrations of  $NH_4^+/NH_3$ ,  $H_2CO_3/HCO_3/CO_3^{2-}$  and volatile fatty acids (VFA) are important factors governing the pH of slurry. Increasing the VFA concentration results in a deceased slurry pH and increasing the total ammonium concentration results in an increased slurry pH. In our studies, reductions of slurry pH were obtained by changing the compositions of urine and/or faeces by dietary composition.

The pH of faeces and slurry was significantly reduced when more NSP was included in the diet of pigs. Increasing the intake of NSP increased the VFA concentrations in faeces and slurry linearly during storage, resulting in a lower pH of faeces and slurry. The pH of slurry lowered by 0.12 unit and ammonia emission by about 5.4% for each 100 g/d increase in dietary NSP intake (Chapter 4). For each 5% increase of sugar beet pulp silage in the diet, total VFA concentration in slurry increased about 25%, the pH of slurry lowered by 0.5 unit and ammonia emission reduced by 15% (Chapter 6).

Influencing the pH of urine can also reduce the pH of slurry. Chapter 7 describes the effects of dEB and acidifying salts on the pH of urine and slurry and on ammonia emission. Lowering dEB from 320 to 100 meq/kg DM of feed reduced urinary pH by about 0.46 unit, slurry pH by about 0.14 unit and ammonia emission by 11%. Adding Ca-benzoate,  $CaCl_2$  or  $CaSO_4$  to the diet instead of  $CaCO_3$ , reduced urinary pH by respectively 1.8, 1.66 and 1.61 units. Slurry pH reduced by respectively 1.53, 0.86 and 0.82 units and ammonia emission by 54, 33 and 30%. Acidity of urine is the result of renal regulation of the acid-base balance in the animal, which in turn is influenced by the acid-base balance of the diet.

The pH of slurry can also be reduced by simultaneously influencing both the pH of urine and of faeces. Dietary manipulation of dEB and NSP levels (Chapters 2 and 3) showed very clear effects on the pH of slurry and on ammonia emission. Reducing dEB from 250 to 120 meq/kg in feed and increasing dietary NSP from 13 to 31% lowered the pH of slurry by about 0.8-0.9 unit and ammonia emission by about 52-53%. This reduction was obtained by the adjusting dietary ingredients commonly used in commercial pig feeding.

#### Ammonia emission and dietary protein level

In Chapter 8 an experiment is described in which the effect of dietary crude protein level on nitrogen excretion and ammonia emission was evaluated in vitro and in vivo. Lowering dietary protein from 16.5% to 14.5 and 12.5% and supplementing essential amino acids (lysine, methionine, threonine and tryptophan) reduced nitrogen excretion in urine but not in faeces. For each 10 g/kg reduction of dietary protein, total nitrogen excretion was reduced by 9.0% and urinary nitrogen by 11.3%. The reduction of urinary nitrogen resulted in the slurry ammonium content reducing by 10.8% (0.75 g/kg slurry) and ammonia emission by 10%, measured in vitro and by 12.5%, measured in vivo.

Good agreement was found between the ammonia emissions measured in vitro and the ammonia emissions measured in vivo. Reducing dietary protein from 16.5% to 12.5% did not affect animal weight gain, feed conversion efficiency or carcass yield characteristics. No interaction effects on the ammonia emission were found between dietary crude protein content and climate factors (temperature and air exchange rate).

#### **Prospects and general conclusions**

The high number and density of the pig population on one hand and inappropriate feeding on the other hand are the primary causes of the high ammonia emissions from pig production systems. Nutrition can substantially contribute to a reduction of ammonia emission in different ways. A good way to obtain a balance between production and environment is to better attune nutrition to the pigs' requirement. Our studies show that feeding influences the rate ammonia is released from pig slurry. The difference in ammonia emission depends not only on the nitrogen components in the diet but also on the non-nitrogen compounds such as nonstarch polysaccharides and minerals. Altering dietary composition changes slurry characteristics, resulting in lower ammonia emissions. Our studies found that the ammonia emission from slurry can be reduced by about 50% depending on the types of diets. If all different solutions were combined even greater reduction might be possible. Generally, in our study, diets were altered by adjusting ingredients widely available for practical pig feeding. Most of them are cheap by-products from human food industries. Using these by-products instead of grains will therefore not only be beneficial for the environment, but will probably also reduce feed costs.

This thesis shows that ammonia emission from pig slurry can be reduced in a relatively simple way by altering dietary composition. This approach can reduce the detrimental effect of pig production on the environment at relatively low costs. Combining feeding measures with the simple housing techniques, already being implemented seems to be the best approach, for the coming years, to reach the major objective of reducing the ammonia emission from pig production into the environment.

150

## Samenvatting

Ammoniakemissie vanuit de varkenshouderij is ongewenst. Deze emissie vermindert de bemestingswaarde van de geproduceerde mest en leidt tot ongewenste veranderingen van natuurlijke ecosystemen. Verschillende factoren beïnvloeden de emissie van ammoniak. De ammoniakconcentratie en de pH van de mest zijn twee van de belangrijkste factoren. De doelstelling van deze studie is te onderzoeken hoe deze twee factoren via voeding van het varken zodanig kunnen worden beïnvloed dat de ammoniakemissie uit de mengmest wordt gereduceerd. De volgende drie mogelijkheden werden onderzocht om dit te bereiken: 1) verschuiving van de stikstofuitscheiding via de vluchtige vorm in de urine, naar de gebonden vorm in de feces; 2) verlaging van de pH van urine en/of feces om de pH van de mengmest te verlagen; 3) verlaging van de totale stikstofuitscheiding door het varken door een lagere stikstofopname. Het onderzoek werd verricht bij vleesvarkens. De belangrijkste uitgangspunten van dit project waren dat deze manieren om de ammoniakemissie te reduceren niet mogen leiden tot negatieve gevolgen voor de diergezondheid of voor de productieresultaten. Daarnaast mogen er geen verschuivingen optreden in de richting van andere milieu-effecten.

In hoofdstuk 1 wordt de doelstelling van deze studie beschreven in het licht van de probleemstelling. In de hoofdstukken 2 en 3 wordt een vooronderzoek beschreven waarin voeders zijn samengesteld die vooral verschilden in gehalten aan "Niet Zetmeel Koolhydraten (NZK)" en in de elektrolytenbalans (dEB = Na + K - Cl). Het effect van deze factoren op het stikstofuitscheidingspatroon, de pH en ammoniumconcentratie van de mengmest en op de uiteindelijke ammoniakemissie zijn bepaald. In het algemeen zijn bijproducten van de humane voedingsindustrie relatief goedkope grondstoffen voor varkensvoer. Vaak hebben deze bijproducten een hoog gehalte aan NZK. In drie experimenten werd het effect van deze bijproducten in het varkensvoer op de ammoniakemissie onderzocht. In het eerste experiment, beschreven in hoofdstuk 4, werd het effect van bron en niveau van NZK op de pH van de feces en de mengmest en op de ammoniakemissie gekwantificeerd. In deze studie werd ontsloten maïszetmeel op drie niveaus vervangen door kokosschroot, sojahullen of gedroogde suikerbietenpulp. Het tweede experiment werd verricht in Vietnam met lokale bijproducten: kokosschroot, rijstzemelen en bierborstel (hoofdstuk 5). Het derde experiment werd gedaan met suikerbietenpulpsilage (SBPS), één van de belangrijkste bijproducten van de voedingsindustrie in Nederland (hoofdstuk 6). In het onderzoek dat beschreven is in de hoofdstukken 4 tot en met 6 was de belangrijkste doelstelling om de ammoniakernissie te reduceren via een verlaging van de pH van de feces. De hypothese was dat een hoger gehalte aan NZK in het voer de vorming van vluchtige vetzuren in de dikke darm van varkens en in de mengmest gedurende de opslag bevordert, resulterend in een lagere ammoniakemissie.

De pH van de urine zou beïnvloed kunnen worden door veranderingen aan te brengen in dEB van het voer en het soort Ca-zout dat toegevoegd wordt aan het voer. Het onderzoek dat beschreven wordt in hoofdstuk 7 laat het effect zien van dEB-niveau en het gehalte en soort Ca-zout (CaSO<sub>4</sub>, CaCl<sub>2</sub> of Cabenzoaat) in het voer op de pH van de urine en mengmest en toont het effect op de ammoniakemissie.

Verlaging van het ammoniumgehalte van de mengmest kan de ammoniakemissie reduceren. Het eerste onderzoeksspoor om dit te bereiken was door een verschuiving te bewerkstelligen van de excretie van stikstof in de urine naar de stikstof in de feces. Dit onderzoek wordt beschreven in de hoofdstukken 2 en 5. In verschillende studies is het effect van de stikstofopname op de stikstofexcretie onderzocht. Heel verschillende resultaten werden gevonden ten aanzien van de relatie tussen stikstofopname en ammoniakemissie. In het onderzoek, beschreven in hoofdstuk 8, is deze relatie vastgelegd onder zeer gecontroleerde omstandigheden. Verder werd onderzocht of het effect van het eiwitgehalte van het voer op de ammoniakemissie werd beïnvloed door stalklimaatsfactoren.

Alle experimenten beschreven in dit proefschrift, op twee na, werden uitgevoerd bij varkens op balanskooien. Het onderzoek met SBPS (hoofdstuk 6) werd uitgevoerd met groepen varkens in klimaatcellen. Het experiment met verschillende eiwitgehalten in het voer (hoofdstuk 8) werd zowel uitgevoerd met varkens op balanskooien, als met varkens in een praktijkstal. De ammoniakemissies werden steeds in vitro gemeten, in een laboratoriumopstelling. De feces en urine, die gescheiden opgevangen waren op de balanskooien, werden gemengd tot mengmest en representatieve monsters werden gebruikt voor de in vitro ammoniakemissie-metingen. De mengmest geproduceerd in de klimaatcellen en in de praktijkstal werd ook bemonsterd voor de in vitro metingen. Resultaten van de in vitro metingen werden gevalideerd met metingen uitgevoerd in de praktijkstal (hoofstuk 8).

De mogelijkheden om de ammoniakemissie te reduceren via voedingsmaatregelen en de implicaties hiervan voor milieu en economie worden bediscussieerd in hoofdstuk 9. Tevens worden in dit hoofdstuk de belangrijkste conclusies van dit proefschrift gegeven.

#### Ammoniakemissie en het stikstofuitscheidingspatroon

Stikstof dat niet aangezet wordt in het dier wordt weer uitgescheiden via urine of feces. Via de feces uitgescheiden stikstof is vooral geïncorporeerd in bacterieel eiwit. Eiwit in de mengmest wordt slechts zeer langzaam afgebroken tot ammoniak en andere componenten. Stikstof in de urine wordt vooral uitgescheiden in de vorm van ureum. Ureum wordt heel makkelijk en snel omgezet in ammoniak en kooldioxide. NZK in het voer kan de stikstofuitscheiding via de urine verschuiven naar uitscheiding via de feces. In ons onderzoek werden voeders met verschillende gehalten aan NZK onderzocht. Bij een gelijke stikstofopname vonden we geen verschillen in de totale stikstofuitscheiding. De stikstofaanzet was dus voor de verschillende voeders vergelijkbaar. Het stikstofuitscheidingspatroon verschilde echter sterk tussen de verschillende voeders. Varkens die voer kregen met een hoger NZK-gehalte scheidden minder stikstof uit via de urine en meer via de feces dan de varkens die voer kregen met een lager gehalte aan NZK. We vonden dat bij een toename van de NZK-opname met 100 g/d de verhouding in stikstofuitscheiding tussen urine en feces afnam met 0.6 eenheid, de stikstofuitscheiding via de urine met 9% (2.78 g/d) en de totale ureum-N met 10% (2.60 g/d) (hoofdstuk 2). Deze verandering in stikstofuitscheidingspatroon werd ook gevonden voor de voeders waaraan bijproducten met een hoog gehalte aan NZK waren toegevoegd. In deze voeders werd tapioca vervangen door de NZK rijke

bijproducten kokosschroot, rijstzemelen of bierborstel. Dit resulteerde in een range in NZK-gehalte van 6.8 tot 20.4%. Het voer met bierborstel had het hoogste gehalte aan NZK. Dit voer zorgde voor de hoogste uitscheiding van stikstof via de feces en de laagste uitscheiding via de urine. De stikstofuitscheiding via de urine, vooral in de vorm van ureum, is sterk gerelateerd aan het ammoniumgehalte van de mengmest (hoofdstukken 2 en 5). Een verlaging van het ureumgehalte van de urine verlaagde in ons onderzoek het ammoniumgehalte en daarmee de ammoniakemissie van de mengmest.

#### Ammoniakemissie en de pH van mengmest

De pH van mengmest is een zeer belangrijke invloedsfactor op de ammoniakemissie. Theoretisch wordt de pH bepaald door de concentratie aan waterstofionen in de oplossing. De concentratie van deze ionen hangt vooral af van de anion-cation balans van de mengmest. De concentraties aan  $NH_4^+/NH_3$ ,  $H_2CO_3/CO_3^-$  en vluchtige vetzuren zijn vooral bepalend voor de pH van mengmest. Verhoging van de concentratie aan vluchtige vetzuren en ammonium resulteert in een verlaging, respectievelijk verhoging van de mengmest pH. In onze studies werd een verlaging van de mengmest pH verkregen door de urine- en/of de fecessamenstelling te wijzigen via het voer.

De pH van de feces en van de mengmest werd significant verlaagd bij een hoger gehalte aan NZK in het voer. Het gehalte aan vluchtige vetzuren in feces en mengmest steeg lineair met de opname aan NZK. De pH van de mengmest daalde met 0.12 eenheid en de ammoniakemissie met 5.4% voor iedere 100 g/d extra opname aan NZK (hoofdstuk 4). Voor iedere 5% toename van SBPS in het voer, werd het vluchtig vetzuurgehalte met ca. 25% verhoogd en de pH en de ammoniakemissie van de mengmest met respectievelijk 0.5 eenheid en 15% verlaagd (hoofdstuk 6).

De pH van mengmest kan tevens worden verlaagd door beïnvloeding van de urine-pH. In hoofdstuk 7 worden de effecten beschreven van dEB en zuurvormende zouten op de pH van de urine en mengmest en op de ammoniakemissie. Het verlagen van dEB van 320 tot 100 meq/kg drogestof in het voer gaf een pH verlaging in de urine en mengmest van respectievelijk ongeveer 0,46 en 0,14 eenheid en een verlaging van de ammoniakemissie van 11%. Toevoeging van Ca-benzoaat, CaCl<sub>2</sub> en CaSO<sub>4</sub> aan het voer, in plaats van de gebruikelijke toevoeging van CaCO<sub>3</sub>, verlaagde de urine pH met respectievelijk 1,53, 0,86 en 0,82 eenheden en de ammoniakemissie met respectievelijk 54, 33 en 30%. De zuurgraad van urine is het resultaat van de regulatie door de nieren van het zuur-base-evenwicht in het dier. Dit evenwicht wordt belangrijk beïnvloed door de zuur-basebalans van het voer.

De pH van mengmest kan ook worden verlaagd door simultaan zowel de pH van de urine als van de feces te beïnvloeden. Verandering in het voer van zowel dEB als NZK niveaus (hoofdstukken 2 en 3) liet een belangrijk effect zien op de pH van de mengmest en op de ammoniakemissie. Een verlaging van dEB van 250 naar 120 meq/kg voer in combinatie met een verhoging van het NZK-gehalte van 13 naar 31% gaf een verlaging van de pH van de mengmest met 0.8 - 0.9 eenheid en een verlaging van de ammoniakemissie met 52 - 53%. Deze reductie werd bereikt door de grondstoffen in het voer te

veranderen. Al deze grondstoffen worden regelmatig in de praktische varkensvoeding gebruikt.

#### Ammoniakemissie en eiwitgehalte in het voer

In hoofdstuk 8 worden de resultaten van een experiment beschreven, waarin het effect van het eiwitgehalte in het voer op de stikstofexcretie en de ammoniakemissie in vitro en in vivo is bepaald. Verlaging van het eiwitgehalte in het voer van 16,5 naar 14,5 en 12,5% onder toevoeging van essentiële aminozuren (lysine, methionine, threonine en tryptophaan) gaf een verlaging van de stikstofexcretie via de urine, maar niet via de feces. Voor iedere 10 g/kg verlaging van het voereiwit werd de totale stikstofexcretie verlaagd met 9,0% en de urinestikstof met 11,3%. De verlaging van urinestikstof resulteerde in een verlaging van het ammoniumgehalte van de mengmest van 10,8% (0,75 g/kg mengmest) en in een reductie van de ammoniakemissie van 10,0%, gemeten in vitro, en van 12,5%, gemeten in vivo. De resultaten van de in vitro studie kwamen goed overeen met de resultaten van de in vivo studie. De verlaging van het eiwitgehalte in het voer van 16,5% naar 12,5% had geen gevolgen voor de groei van de dieren, voor de voederconversie of de slachtkwaliteit. Het effect van het eiwitgehalte van het voer op de ammoniakemissie werd niet beïnvloed door de stalklimaatsfactoren temperatuur en luchtsnelheid.

#### Verwachte effecten en algemene conclusies

Het grote aantal varkens op een klein oppervlak in combinatie met inadequate voeding is de belangrijkste oorzaak van de hoge ammoniakemissie vanuit de varkenshouderij. Voeding kan via verschillende sporen een belangrijke bijdrage leveren om de ammoniakemissie te reduceren. Een betere afstemming tussen opname en behoefte is een belangrijke weg om een betere balans te krijgen tussen productie en milieu. Uit onze studie blijkt dat voeding een belangrijke invloed heeft op de ammoniakemissie uit varkensmengmest. De verschillen in ammoniakemissie worden niet alleen beïnvloed door de stikstofcomponenten in het voer, maar tevens door de niet-stikstofcomponenten, zoals niet-zetmeel koolhydraten en mineralen. Verandering van de voersamenstelling veroorzaakt een verandering van de chemische samenstelling van de mengmest, resulterend in een hogere of lagere emissie van ammoniak. Afhankelijk van de mate en het type van voeraanpassing, kan de ammoniakemissie tot ongeveer 50% worden gereduceerd. Wanneer verschillende typen voeraanpassingen worden gecombineerd, kunnen zelfs hogere reducties worden verkregen. In het algemeen hebben we in onze studie het voer aangepast door verandering van de grondstoffensamenstelling. De gebruikte grondstoffen zijn, in het algemeen, ruim beschikbaar voor gebruik in varkensvoer. De meeste zijn relatief goedkope bijproducten uit de humane voedingsindustrie. Vervangen van graan in varkensvoer door deze bijproducten is daarom waarschijnlijk niet alleen gunstig voor het milieu, maar zal tevens de voerkosten kunnen verlagen.

Dit proefschrift laat zien dat door aanpassing van de voersamenstelling de ammoniakemissie uit varkensmengmest op een effectieve manier kan worden gereduceerd, zonder dat dit nadelige gevolgen hoeft te hebben voor de productieresultaten van de dieren. De milieubelasting kan op deze manier worden verminderd tegen relatief geringe kosten. Combinatie van de voedingsmaatregelen, zoals genoemd in dit proefschrift, met huisvestingsmaatregelen, zoals die op dit moment al in gebruik zijn, lijkt de beste weg om op korte en middellange termijn een belangrijke reductie van de ammoniakemissie uit de varkenshouderij te bewerkstelligen.

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#### **CURICULUMVITAE**

Truong Thanh Canh was born August 20, 1955 in Canh Duong, Quang Binh, Vietnam. In 1979 he received his BSc. diploma from Agricultural University No. 2 (Vietnam), with a specialisation in Animal Husbandry. In 1979-1980 he studied Biochemistry in Hanoi University. From 1979 he was lecturer of Agricultural University No. 2 (presently named Hue University of Agriculture and Forestry), Department of Animal Nutrition and Chemistry and latterly He become the head of this Department. In 1987 he studied animal feed analyses in the Department of Animal Nutrition, Wageningen Agricultural University. In 1993 he received his Master degree in Animal Nutrition in Wageningen Agricultural University. From 1994-1998 he has been working as a researcher of the Department of Livestock Engineering, Institute of Agricultural and Environmental Engineering (IMAG-DLO), and the Department of Animal Nutrition, Wageningen Agricultural University, The Netherlands.